14

RELATION OF CRYSTALLINE STYLE FUNCTION TO FOOD AVAILABILITY AND ENVIRONMENTAL CONDITIONS IN SOUTH AFRICAN MUSSELS

by

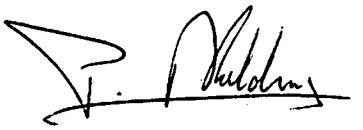
Peter John Fielding

Submitted for the degree of Doctor of Philosophy in the University of Cape Town

August 1987

DECLARATION

This thesis reports the results of original research which I have carried out in the Department of Zoology, University of Cape Town between 1982 and 1987. None of it has been submitted in whole or in part for any other degree and any technical assistance I have received is fully acknowledged. Chapter III was carried out in collaboration with Dr. M.I. Lucas and J.M. Harris.



P.J. Fielding

For Bridget, my parents, Marko and Ant

CONTENTS

ABSTRACT

The mussels Choromytilus meridionalis, Mytilus galloprovincialis, Perna perna and Aulacomya ater are important and often dominant organisms on the littoral and sublittoral shores of the South Africa. M. galloprovincialis has only recently been identified as a seperate species, but a considerable body of information exists on the physiology and energetics of C. meridionalis, P. perna and A. ater. However, it is not clear what factors determine the different intertidal and geographic distributions of these mytilids. Work in the kelp beds has shown that the energy budgets of mussels depends on the utilisation of particulate material from both kelp and phytoplankton production, which have very different structural complexities and biochemical compositions. There is very little information on the digestive enzymes of South African mussels. The activity of these enzymes will be an important factor affecting the ability of the mussels to effectively utilise a food resource, and this may be a mechanism which partially accounts for their differing distributions. This work examines the quantitative and qualitative nature of the suspended particulate food resource at the boundary of the east and west coast mussel ranges, the differences in the digestive enzyme activities of the four mussel species that might utilise the food resource, and the animals' abilities to digest this particulate material.

A typical kelp bed system on the west coast of the Cape Peninsula, South Africa, is characterized by pulses of upwelling caused by offshore southeasterly winds in the summer months and onshore northwesterly winds in winter. Large winter swells cause an increase in the fragmentation of kelp macrophyte frond tips, and particulate suspensoids have mean C:N ratios of 9,90 in winter compared with 8,14 in summer, indicating that a large part of the winter particulate fraction is derived from kelp material (C:N = 11,70 - 17,28) rather than phytoplankton (C:N = 6,40 - 7,30). The water column particulate load is most closely correlated with wind and temperature in summer, and wind and swell height in winter. Particulate organic carbon and nitrogen available to mussels was 335 $\mu gC.l^{-1}$ and 41 $\mu gN.l^{-1}$ in summer and 469 $\mu gC.l^{-1}$ and 48 $\mu gN.l^{-1}$ in winter. Carbon requirements of *C. meridionalis*, *M. galloprovincialis*, *P. perna* and *A. ater* indicate that the carbon demand of all four species could be met in summer and winter by the particulate organic carbon component, despite summer upwelling events which may remove particulates from the area. The particulate organic nitrogen resource can meet the nitrogen requirements of *P. perna* and *A. ater*, but in summer this resource may be limiting for *C. meridionalis* and *M. galloprovincialis*.

High Performance Liquid Chromatography was used to examine the importance of phytoplankton as a food resource in the kelp bed system and qualitative changes taking place in the

suspended particulate fraction. Mean chlorophyll *a* concentrations were 1,64 $\mu g.l^{-1}$ in summer and 1,78 $\mu g.l^{-1}$ in winter, and were significantly lower than spectrophotometric determinations. The occurrence of fucoxanthin and chlorophyll *c* in more than 90% of winter samples indicated that much of the particulate component in winter was of diatom and kelp origin. Summer phytoplankton blooms were not dominated by diatoms. Mean organic carbon to chlorophyll *a* ratios were 344 in summer and 742 in winter. These values, together with the poor correlations between organic carbon and nitrogen, and chlorophyll *a*, indicated that throughout the year, much of the particulate oganic carbon and nitrogen available to filter feeders in the system, was of a detrital nature.

The activities of bivalve crystalline style enzymes used in the extracellular digestion of ingested material have commonly been measured by the Nelson-Somogyi and Bernfeld assays for reducing sugars. During quantitative assays using glucose for calibration purposes, there is a two to sevenfold discrepancy between the two methods. It seems likely that the quantitative discrepancy between the two methods can be acounted for by variability in colour response to different end products. When the appropriate calibration standard is used the two methods are in close agreement. These two reducing sugar assays have previously been used with an inappropriate standard (glucose) to make quantitative estimates of reducing sugar release in order to calculate the

energetic gain from crystalline style activity. It seems likely that such energy balance studies are in error.

The rates of reducing sugar release from commercial polysaccharide substrates by the major crystalline style and digestive gland carbohydrases of C. meridionalis, M. galloprovincialis, P. perna and A. ater were examined in relation to habitat and distribution of the species. Although temperature coefficients for crystalline style α-amylase activity in all four species were very similar (1,99 -2,81), there were major differences in digestive gland α-amylase temperature coefficients, which ranged from 1,97 and 1,99 for C. meridionalis and M. galloprovincialis, to 1.00 for P. perna. P. perna therefore relies exclusively on crystalline style enzyme activity to combat increased metabolic demands resulting from higher temperatures.

A number of α-amylase and laminarinase isozymes of differing molecular weights were present in the style and digestive gland of the four species of mussel. M. galloprovincialis had more isozymes of α-amylase than any of the other mussel species. Style α-amylase protein profiles of M. galloprovincialis included a peak with a "specific activity" of 3142,21 mg maltose.mg protein.h^{-1}, which was more than three times that of any α-amylase activity in the other species. This may explain the recent success of this species in colonizing the west coast of South Africa.

Saccharogenic rates of M. galloprovincialis and P. perna were 10,51 and 10,54 mg maltose.mg protein.h^{-1} respectively while those for C. meridionalis and A. ater ranged between 4,62 and 6,54 mg maltose.mg protein.h^{-1}. Higher rates of saccharogenesis allow M. galloprovincialis and P. perna to colonize the mid intertidal zone where feeding times are reduced, while C. meridionalis and A. ater are restricted to the low intertidal. Total saccharogenesis by the digestive gland of the west coast species C. meridionalis, M. galloprovincialis and A. ater was twice that of the south and east coast P. perna. The predominantly detrital food resources of the west coast may require extensive processing by the digestive gland for effective hydrolysis, and therefore P. perna is prevented from successfully colonizing the west coast. In C. meridionalis and A. ater, which are closely associated with the kelp beds, laminarinase activity accounted for 57% of total style saccharogenesis, and for 70% of total digestive gland saccharogenesis in A. ater. Laminarinase accounted for approximately 38% of total style saccharogenesis in M. galloprovincialis and P. perna. There is therefore some specialization for a laminarin diet in the kelp bed mussels.

Total hydrolytic potential of M. galloprovincialis digestive enzymes was 182,50 mgC.h^{-1} compared with values of 60,43-139,12 mgC.h^{-1} for the other mussel species and this high potential carbon production may make

M. galloprovincialis a very successful competitor on the South African coastline.

Although mussel digestive enzymes readily liberate reducing sugars from commercial polysaccharide preparations, rates of hydrolsyis of a naturally occurring detrital substrate were very much lower and were similar for all mussel species. On an hourly basis, the crystalline style could meet between 2% and 6% and the digestive gland between 110% and 236% of the mussels' carbon requirements by hydrolysis of detrital material . Style and digestive gland protein would need to be renewed at least once every two hours to meet carbon requirements. Mussel digestive enzymes leached pigments from diatom and flagellate cells in in vitro incubations, but after 8 hours no cell breakdown was observed. Cell breakdown occurred in the mussel gut within 30 minutes. Thus there are considerable differences between rates of digestive enzyme hydrolysis of commercial substrates and of detrital material, and it is evident that in vitro incubations do not duplicate processes in the mussel gut. Bacterial hydrolases or the breakdown of food material by trituration in the gut may facilitate the digestive process in mytilids. The role played by bacteria in the absorption of organic material was investigated.

The absorption efficiency of M. galloprovincialis fed on natural detrital material was 0,27. Subsequent addition of antibiotics to the water eliminated 98% of the gut and style

bacteria without affecting other physiological functions. Absorption efficiencies then declined to 0,16. When gut and style bacterial populations were re-established, absorption efficiencies in the same animals increased to 0,37. Thus bacteria appear to play a significant role in bivalve digestion of a refractory detrital substrate, although it is not clear whether endogenous bacteria or bacteria colonising the particulate material assist in digestion.

Although there are major differences in the digestive enzyme activities of the four mussel species, which appear to be related to their distributions, it is difficult to assess the significance of these differences as a mechanism accounting for the different intertidal and geographic distributions of the species, since bacterial activity and probably mechanical breakdown of particulate material appear to play an important part in the efficiency of utilisation of the food resource.

ACKNOWLEDGEMENTS

I am most grateful for the encouragement and advice of Dr. Peter A. Cook and Dr. Charles L. Griffiths of the Zoology Department. Dr E. Merrifield of Clinical Sciences, Dr S. Churms of the CSIR Carbohydrate Research Unit, Dr. George Lindsey of the Biochemistry Department, Professor Frank Robb and Eric O'Neill of the Microbiology Department, and Professor John Field and Jeanie Stenton-Dozey of the Zoology Department all offered extensive advice, and on occasions the use of equipment. I am also indebted to Professor Richard Newell of IMER, Plymouth for his interest in my work and his ability to brighten the darkest moments. Very special thanks are due to my friends and colleagues Dr. Venetia Stuart, Dr Amos Barkai and Jean Harris for the time and trouble they have taken to discuss aspects of my work with me. Thanks also to Dr. Dave Muir and Dr. Trevor Probyn for information on nutrients and bacterial populations in the kelp bed, to Pedro Monteiro for assistance with the HPLC, to Coleen Moloney, Sheldon Dudley, Carlos Villacastin-Herrero and Patti Wickens for statistical and computing advice, and to Sandi Tolosana for typing. Above all, I must thank Dr. Muffy Seiderer and Dr. Mike Lucas, for without their interest, advice and support, much of this work would not have been possible. I also owe a great deal to my wife Bridget, for typing and drafting, and particularly for her great patience, help and encouragement throughout. I am also very grateful to Jill, Felicity, Rayna, Kishor and Janice for proof-reading

different sections of this work. Mr. W.R.T. Hemsted supervised my use of the CHN analyser and wind data were made available by ESCOM. This work was partly supported by funds from CSIR and partly from ESCOM.

GENERAL INTRODUCTION

The Mollusca are amongst the most widely studied invertebrates in the animal kingdom. A recently published review in six volumes (Wilbur, 1983) describes much of the accumulated research on this group of animals since Sir Maurice Yonge studied molluscan physiology in the 1920s. A great deal of this research has been concentrated on the Bivalvia, and a large body of information exists on the biochemical and physiological processes of this group. With respect to the processes of gains and losses of energy and the efficiencies of energy transformation, Bayne and Newell (1983) review the physiological energetics of marine molluscs and conclude that the combined effects of temperature and food availability have the most profound influences on physiological energetics in the natural habitat.

In suspension feeding bivalves, factors relating to the capture of particulate food have been particularly well studied (for reviews see Bayne, 1976; Winter, 1978; Bayne and Newell, 1983). The mechanics of filtration and movement of filtered particles from the gills to the oesophagus and stomach are well understood in lamellibranch bivalves, as are the grades of organization in the stomach. The basic features of the stomach include a muco-protein crystalline style which rotates against a gastric shield, and a digestive gland which generally surrounds the stomach and communicates with it through a system of ducts. The dissolution of the

style and the consequent release of enzymes is the principal means of extracellular digestion, while the digestive diverticula function mainly as organs of absorption and intracellular digestion (for reviews, see Bayne, 1976; Morton, 1983).

Yokoe and Yasumasu (1964), Sova et al. (1970), Kristensen, (1972a), Gianfreda et al. (1979) and Onishi et al. (1985) describe polysaccharide hydrolase activities in a wide range of marine molluscs. Carbohydrases present in bivalves include α-amylase, laminarinase and cellulase, as well as chitinase (Birkbeck and McHenery, 1984; Smucker and Wright, 1984), fucosidase (Cabezas et al., 1981), alginate lyase (Seiderer et al., 1982) and several glycosidases (Pernas et al., 1981; Mayasich and Smucker, 1986).

However, apart from the studies of Seiderer et al. (1982) and Lucas and Newell (1984), very little information is available on how these enzymes relate to the energetics of the animal. The regulation of the complement of digestive enzymes and their activities forms a mechanism whereby the animal can control the amount of food absorbed during passage through the digestive system (Bayne and Newell, 1983; Gabbott, 1983). It is also possible that the distribution of some bivalves is limited by their ability to adjust these enzyme activities and therefore the absorbed ration, to different conditions.

Clearly, the availability of suitable space for habitat and the quantity of food available will be of major importance in determining distribution. However, where very similar organisms such as different mussel species compete for the same resource, the ability to utilise the resource more efficiently may provide one species with a competitive advantage over the others.

On the South African coastline, four species of mussel occur with overlapping but nevertheless fairly distinctly defined distributions. Their distributions are currently under review (Griffiths, pers. comm.), but the areas of major occurrence are shown in Figure 1 which is reproduced from Grant et al. (1984). Perna perna is largely confined to the south and east coasts bounded by the warm Indian ocean and occurs subtidally and in the mid and low intertidal regions. Choromytilus meridionalis and Mytilus galloprovincialis occur mainly on the cold Atlantic west coast while Aulacomya ater is the dominant bivalve subtidally in the kelp bed systems of the west coast (Velimirov et al., 1977).

The intertidal distribution of C. meridionalis, M. galloprovincialis and A. ater is of some interest. The three species occur in mixed populations in the intertidal zone, but C. meridionalis and M. galloprovincialis generally greatly outnumber A. ater, which tend to be small compared with subtidal animals (Griffiths, 1977; Gardiner, 1980). C. meridionalis occurs in the low intertidal zone and

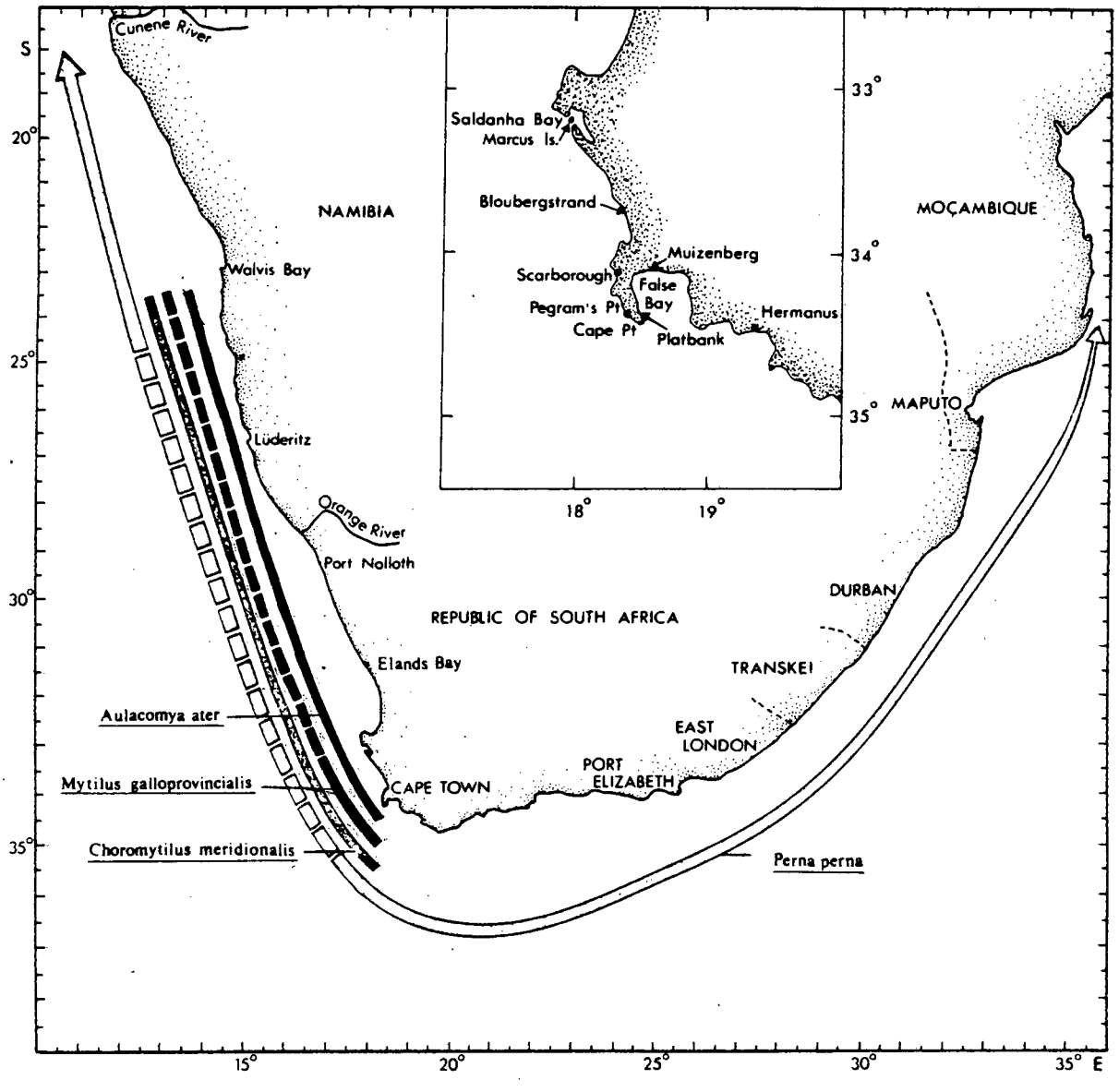


Figure 1. Chart of southern Africa showing the areas of major distribution of C. meridionalis, P. perna, M. galloprovincialis and A. ater, adapted from Grant et al. (1984).

subtidally in the kelp beds (Griffiths, 1981a) while M. galloprovincialis occurs in the mid and low intertidal zone (Grant et al., 1984; pers obs.). M. galloprovincialis has probably only appeared on the South African coastline in the last two decades (Grant and Cherry, 1985) and although it has not been recorded subtidally in the kelp beds it grows well on culture ropes in Saldanha Bay.

At Blouberg Strand on the west coast, where much of the sampling for mussels was done (see Figure 1), M. galloprovincialis grows far higher above chart datum than C. meridionalis, and over the last four years has come to dominate the intertidal area formerly occupied by C. meridionalis (pers. obs). At Elands Bay, 150 km further north, A. ater and C. meridionalis occupied the intertidal zone four years ago but recent inspection showed that these species have been replaced by M. galloprovincialis. Furthermore, the numbers of M. galloprovincialis on the coastline east of Cape Town appear to be increasing (Cherry pers. comm.). Thus in the vicinity of the Cape Peninsula, the distributions of east and west coast mussel species which occupy differing tidal levels on the shore, overlap, and one species (M. galloprovicialis) appears to be extending its range. Slow clearance rates of A. ater (Stuart,1982; Bayne et al., 1984) probably limit this species to the low intertidal and subtidal areas. However, it is not clear why a species such as C. meridionalis, which has a higher weight-specific clearance rate than P. perna (Bayne et al., 1984), is limited

to the low intertidal zone, while P. perna extends into the mid-intertidal zone where feeding times are limited. It is also not clear why P. perna is unable to successfully colonise the kelp dominated western coastline and why the range of M. galloprovincialis should be increasing. On the basis of particle size, there is no partitioning of the food resource among the different mussel species (Stuart and Klumpp, 1984), and in the area of overlap the different species are competing for the same food resource.

In this thesis the hypothesis is tested that the ability of the different mussel species to digest particulate foods with differing efficiencies influences their distribution on the shore. The following work was therefore undertaken to investigate the particulate resources available on the west coast of the Cape Peninsula, and the ability of the different mussel species to exploit this resource. The thesis is divided into three sections. The first section describes the quantitative and qualitative nature of the particulate resource available to filter feeders since it is this which will determine whether the ecosystem can support a filter feeding population, and how appropiate the digestive enzymes are of each of the mussel species. The second section examines mussel crystalline style and digestive gland enzyme activities. Firstly, the methods that have been commonly used to assess carbohydrase activities in molluscs are critically evaluated and secondly, the ability of C. meridionalis, M. galloprovincialis, P. perna and A. ater style and

digestive gland carbohydrases to hydrolyse purified substrates is described and related to the energy requirements, diets and distributions of the species. In the third section of the thesis, the ability of these carbohydrases to release sufficient energy from the hydrolysis of naturally occurring particulate material is examined and the role of bacteria in the digestion of natural food sources is investigated.

SECTION I

THE QUANTITATIVE AND QUALITATIVE NATURE OF PARTICULATE RESOURCES AVAILABLE TO KELP BED FILTER FEEDERS.

The ability of an ecosystem to support a filter feeding community will be greatly influenced by the food available. The quantity of the food resource may vary with time and environmental conditions, thus affecting the capacity of the system to support particular filter feeding components, and changes in the quality of the food resource will change the energy gained from the filtering process. Thus the aim of this section is to quantify and qualitatively characterize the exact nature of the particulate resource, since it is this which will largely determine the effectiveness of the digestive enzymes of the filter feeding species that utilize the resource.

CHAPTER I

CARBON AND NITROGEN RESOURCES AVAILABLE TO KELP BED FILTER FEEDERS

INTRODUCTION

The past decade has seen considerable research effort aimed at understanding the ecology of kelp bed systems, such as those on the west coast of South Africa. Many of the data described below were compiled for Oudekraal ($33^{o}59'S$ $18^{o}21'E$), a typical kelp bed system on the west coast of the Cape Peninsula, South Africa, where an upwelling plume is a semi-permanent feature of the area during summer. Initially, studies provided data on physical and chemical parameters, water movements into and out of the system (Field et al., 1980; Jury, 1980; Carter, 1982; Brown and Field, 1986;) standing stocks and growth rates of kelp (Mann et al., 1979; Dieckmann, 1980; Jarman and Carter, 1981), and phytoplankton biomass and production (Borchers and Field, 1981; Carter, 1982; Barlow, 1982; Brown, 1984; Brown and Field, 1985, 1986).

Further studies centered on the fate of kelp production and the composition of the particulate and soluble components of this production, and its decomposition by micro-organisms in the kelp bed community (Newell et al, 1980a, 1981, 1982; Velimirov, 1980; Jarman and Carter, 1981; Linley et al., 1981; Lucas et al., 1981; Newell and Lucas, 1981; Stuart et al., 1981; Koop et al., 1982). The succession and energetic efficiency of microbes colonizing kelp particulate debris (Linley et al., 1981; Newell and Lucas, 1981; Stuart et al., 1981; Koop et al., 1982; Linley and Field, 1982),

phytoplankton (Newell et al., 1981) and faecal material from the consumer community (Stuart et al., 1982b) have also been reported.

Extensive surveys were made of the consumer communities inhabiting the kelp beds on the South African west coast (Field et al., 1977; Velimirov et al., 1977;), and various studies have been carried out on the standing stocks, production and energetics of the most important consumer organisms in the kelp bed (Velimirov et al., 1977; Griffiths and King, 1979a,b; Greenwood, 1980; Griffiths, 1980a,b; Stuart, 1982; Klumpp, 1984; Stuart and Klumpp, 1984;).

Kelp bed fauna is dominated by filter feeders which comprise 72% of total standing stocks, accounting for 77% of total production and 84% of total consumption (Newell et al., 1982). Several authors have described the filtration and assimilation efficiencies of the mussels, sponges and ascidians that make up 64% of the filter feeding component (Griffiths and King, 1979a; Griffiths, 1980a,b; Stuart, 1982; Klumpp, 1984; Stuart and Klumpp, 1984).

Since a good deal is known about the production and population energy requirements of the kelp bed community, it has been possible to describe the energy balance of the community (Newell et al., 1982). The carbon and nitrogen flow through the system arising from detrital, phytoplankton and bacterial sources has also been quantified (Newell and Field,

1983). The different trophic pathways under upwelling and downwelling conditions have been modelled by Wulff and Field (1983). Newell and Field (1983) concluded that the consumer community depends on the utilization of carbon directly from the sources of primary production rather than via decomposer organisms, which could contribute only 9% of the estimated carbon requirements. They calculated that sources of primary production could meet 87,6% of the estimated nitrogen requirements of the consumer organisms, and bacteria could supply 59-73% of the nitrogen requirements, although the contribution by bacteria to the nitrogen budget of different organisms will depend on how efficiently they can be filtered from the water column (Stuart and Klumpp, 1984; Lucas et al., 1987). Estimates of carbon and nitrogen resources available to the consumers were obtained indirectly from measurements of primary production and C:N ratios of macrophytes and phytoplankton in the system (Newell et al., 1982; Newell and Field, 1983). However these sources may not provide a realistic estimate of the particulate food resources available to filter feeders. The system is subjected to pulses of upwelling generated by strong southeasterly winds in the spring and summer, followed by periods of downwelling (Andrews and Cram, 1969; Andrews and Hutchings, 1980; Field et al, 1980), which can result in a very rapid export and import of organic material and nutrients. During strong upwelling and downwelling regimes the water volume of a kelp bed is exchanged between three and seven times a day (Field et al., 1980). Thus although phytoplankton and macrophyte

production in the kelp bed can be measured, during conditions of upwelling and export, the particulate organic load in the water column may be too low to sustain filter feeders, while during downwelling and the import of organic material from the offshore region, the particulate load may be much higher than would be expected from production studies. Since the upwelling season lasts approximately eight months of the year (Wulff and Field, 1983), it is possible that filter feeding organisms are nutritionally stressed by low levels of particulate material in the water column, in spite of very high primary production rates recorded in the area (Dieckmann, 1980; Borchers and Field, 1981; Carter, 1982).

Carter (1982) has reservations about annual phytoplankton production estimates because of the acccumulated errors in extrapolating daily and seasonal rates to annual rates in such a variable system. Brown and Field (1985) describe large scale diel fluctuations in phytoplankton productivity. In an attempt to characterize short term fluctuations in a highly variable system and to assess the carbon and nitrogen resources available to the filter feeding community in a kelp bed system, an intensive sampling programme was carried out for two months in summer and one month in winter. The object of this was to determine the effect of physical parameters on particulate load in the water column and to assess whether these food resources balanced the requirements of different mussel species. A comparison could be made between food

resources estimated from primary production studies and those actually measured in the water column.

MATERIALS AND METHODS

Sampling procedure: Particulate carbon and nitrogen

Water samples were collected daily at 0800 h from the shore at Oudekraal for 52 days in November/December 1984, which is defined as the main spring/summer upwelling season (Andrews and Hutchings, 1980), and 31 days in June/July 1985 (winter). The water was passed through a 200 µm mesh to remove large particles, and a measured volume of between 100 ml and 900 ml, depending on particulate load, was immediately filtered through a pre-ashed (400^{o}C for 6h) 25mm Whatman GF/F filter by gentle hand pump filtration. Filters were oven dried at 50^{o}C for three days and combusted in a Heraeus CHN-Mikro Universal combustion analyser calibrated with cyclohexanone (Monar, 1972). As a control, unused but pre-ashed filter papers were combusted and the carbon and nitrogen present were subtracted from experimental results. The net carbon and nitrogen values for each filter paper were expressed as $\mu g.l^{-1}$ and compared with the carbon and nitrogen requirements of four mussel species and primary production estimates of water column particulate carbon and nitrogen.

Environmental parameters

Sea temperature was measured at the time of sample collection and a visual estimate made of swell height. Average daily wind speed and direction at a height 10 m above the sea surface were obtained from the Koeberg weather station about 10 km north of the sample site. The longshore wind component was calculated according to the upwelling index

$$Vt = U(\cos \theta - 160)$$

where U = wind speed, θ = wind direction and 160 is the orientation of the Cape west coast (Jury, 1980). These were plotted on a scale of +10 to -10 where 0 represents the boundary between upwelling (+) and downwelling (-). Multiple stepwise correlation analysis was performed using Statpro (Wadworths), to assess the influence of temperature, wind and swell on particulate carbon and nitrogen levels in the water column. Analysis was terminated when no additional variable was correlated below the 0,05 significance level.

C:N ratios

To calculate ambient C:N ratios for suspended particles, the material accumulating on the 0,45 µm intake filter of the Sea Fisheries Research Institute aquarium pump, which draws water directly from the sea 5 km from Oudekraal, was backwashed into a container and the contents centrifuged (7000 x g for 15 mins) to precipitate organic matter. The precipitate was lyophilized and three weighed samples were combusted in the CHN analyser. This procedure was repeated on

three occasions. This material was considered to be a mixture of phytoplankton and detritus. During the summer sampling, tips of mature kelp fronds were removed, lyophilized, pooled, pulverized and three weighed samples were subjected to CHN analysis.

RESULTS AND DISCUSSION

Summer and winter means of all the variables measured are summarized in Table 1.

Temperature and Wind

In Figure 2 A the longshore component of windstress shows that during the summer months, the Oudekraal area was characterized by strong southeasterly winds which caused upwelling of cold water into the system (Figure 2 B). When southerly winds relaxed or changed to onshore northerly/northwesterly winds, stabilization of the water column or downwelling occurred. This was accompanied by a rise in water temperature. In winter onshore northerly winds prevailed, little upwelling occurred and sea temperatures remained fairly stable. This is in agreement with previous studies such as those of Field et al., (1980), Andrews and Hutchings (1980), Dieckmann (1980), Carter (1982), and Brown and Field (1985, 1986).

TABLE 1

Summary of summer and winter means of all variables measured. Significant seasonal differences are also shown.

Variable	Summer(±SD)	Winter(±SD)	Significant difference (t test)
Temperature (°C)	12,1 (± 1,7)	15,2 (± 1,0)	+ ($p < 0,01$)
Wind (Upwelling Index)	2,45 (± 3,76)	-0,93 (± 3,29)	+ ($p < 0,01$)
Swell height (m)	0,49 (± 0,66)	1,25 (± 0,92)	+ ($p < 0,01$)
Carbon $\mu g.l^{-1}$	533 (± 242)	764 (± 425)	+ ($p < 0,01$)
Nitrogen $\mu g.l^{-1}$	66 (± 29)	79 (± 43)	- ($p > 0,05$)
C:N ratio	8,14 (± 1,6)	9,90 (± 2,08)	+ ($p < 0,01$)

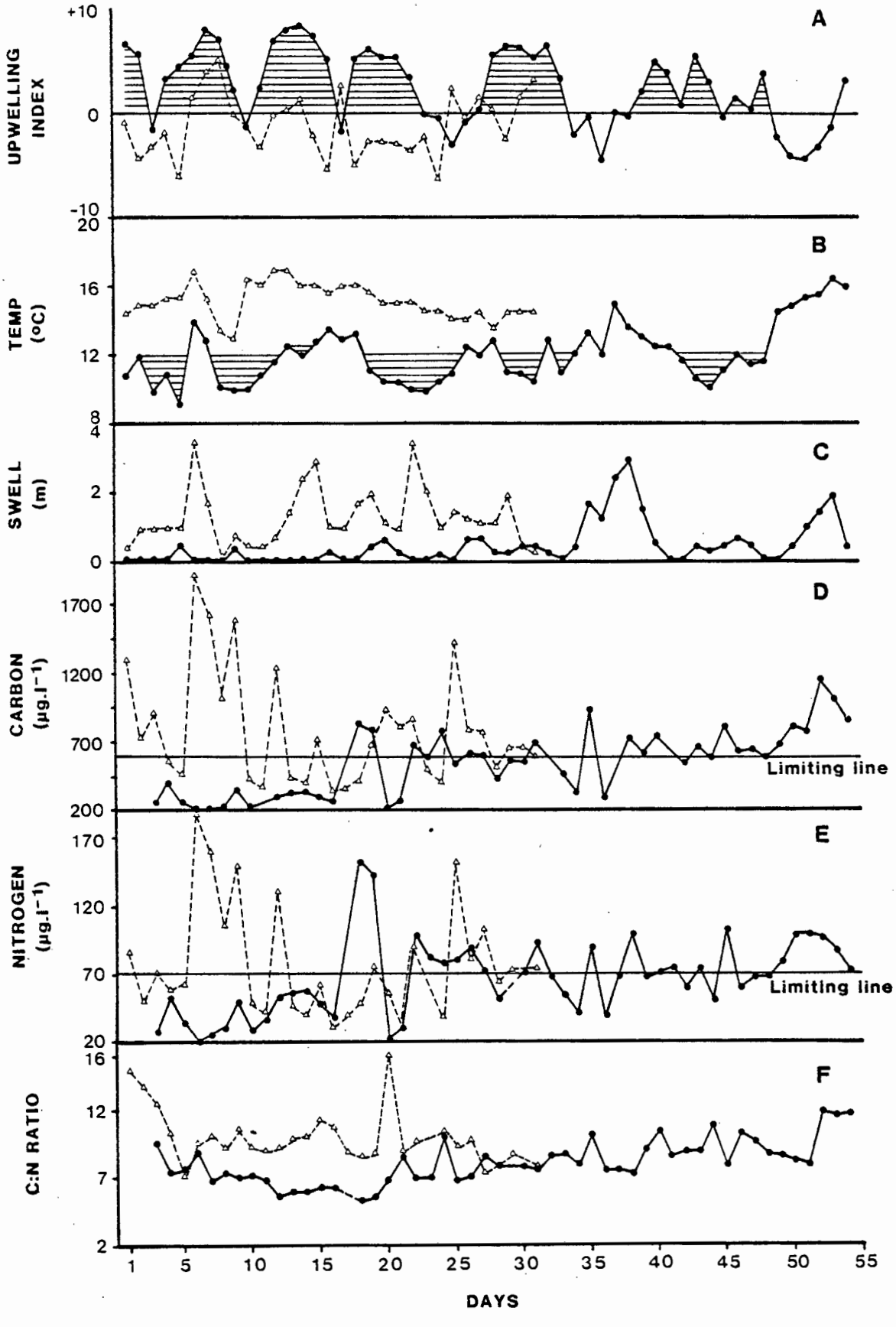


Figure 2. Summer (●——●) and Winter (△---△) variables in the kelp bed.

Figure 2 A and B clearly shows the pulsed nature of the summer southerly winds and the consequent upwelling processes (hatched areas). Southeasterly winds blew for periods of approximately five days, after which wind stress relaxed before the next pulse. Strong offshore winds in summer were usually accompanied by a drop in sea temperature which generally lagged the wind factor by a day (Figure 2 A and B). Five major pulses of southeasterly winds occurred, between days 4-9, 11-16, 18-22, 27-33 and 39-44. The second pulse was not strongly marked by a temperature drop because of solar heating, but high nitrate concentrations recorded in water samples taken during this period indicated that upwelling had occurred (Muir, 1986). When strong northwesterly winds blew, such as on days 34-36 and 49-53, sea temperatures rose rapidly to winter levels. Although the prevailing wind was northwesterly in winter, on days 6-8 strong southeasterly winds blew and a sharp drop in temperatures occurred (Figure 2 A and B). Thus sea temperatures were closely associated with the wind regime.

Mean summer sea temperature for the sampling period was 12,1 ($\pm$ 1,7)^{o}C, which is close to the mean sea surface temperature of 12,7^{o}C for the 1977/78 and 1978/79 upwelling season (Brown and Field, 1986). Mean winter sea temperature was 15,2 ($\pm$ 1,0)^{o}C which is higher than the 13,2^{o}C of Brown and Field (1986). Seasonal differences in sea temperature are significant (t-test, $p < 0,01$).

Wave action

Strong northwesterly winds generated large swells (Figure 2 A and C) and these may reach a height of 5-7 m (Velimirov et al., 1977; Field et al., 1980), producing considerable turbulence in the inshore area. The largest swells recorded during the sampling period were 3,5 m. In summer wave action is much reduced, and long periods of calm seas may occur (Figure 2 C), although even in summer, northwesterly winds will rapidly increase swell size (see Days 3-4, 25-26, 34-37 and 49-53 in Figure 2 A and C). Mean winter swell height was 1,25 (± 0,92) m while the summer mean was 0,49 (±0,66) m. Seasonal differences in swell height are significant (t-test $p < 0,01$).

Particulate load

It has been generally accepted that during upwelling, when offshore winds move surface waters away from the coast, particulate and chlorophyll concentrations decline to very low levels (Field et al., 1980; Brown, 1981; Barlow, 1982; Carter, 1982; Brown and Field, 1985, 1986). However, Figure 2 D and E shows that during the main upwelling season, even though strong southeasterly winds blow at regular intervals (Figure 2 A), levels of particulate carbon and nitrogen in the water column, although lower, are less variable than in winter. Mean summer chlorophyll a concentration was 1,64 $\mu g.l^{-1}$ (Chapter II) and seldom declined to the very low values recorded further offshore in newly upwelled water

(Andrews and Hutchings, 1980; Barlow, 1982; Brown and Field, 1986; Lucas et al., 1986). In the inshore region, because of the sheltering effect of the mountainous Cape Peninsula and increasing bottom drag, rates of upwelling are lower than further offshore (Andrews and Hutchings, 1980), and the southeasterly summer winds may therefore not be overridingly important in regulating the food available to inshore primary consumers. Field et al. (1980) have shown that during upwelling, currents in the water column are offshore at the surface and onshore on the bottom, and bottom currents may reach speeds of 10 $cm.sec^{-1}$ or more during active upwelling. These onshore bottom currents may resuspend enough material to maintain particulate organic levels in the water column (Figure 2 D and E).

It has also been stated that there is a large export of organic matter by wind driven water currents in the upwelling season during rough weather, because of the fragmentation of kelp and the resuspension of bottom material (Field et al., 1980; Newell et al., 1982). Figure 2 C demonstrates that large waves were not a common phenomenon in summer, and on only 3 days out of 54 was the swell height > 2m. Thus the importance of resuspension and export of organic matter by the combined action of waves and offshore winds should not be overestimated in the context of kelp bed dynamics. In winter, onshore winds may result in a net import of offshore detritus and phytoplankton to the inshore region (Field et al., 1980) but more importantly, the large swells generated by these

winds greatly increase kelp plant erosion and destruction, as well as the resuspension of particles that have sunk to the sea bed.

a) Carbon

With the import of detrital/phytoplankton particles by onshore winds and the increased erosion and destruction of kelp by strong wave action, higher particulate carbon levels might be expected in winter than in summer, and this is evident from Figure 2 D. Mean daily particulate carbon levels were 764 ($\pm$ 425) $\mu gC.l^{-1}$) in winter and 533 ($\pm$ 242) $\mu gC.l^{-1}$ in summer. These values compare well with those reported by Stuart and Klumpp (1984) and Seiderer and Newell (1985). Seasonal differences were significant (t-test, $p < 0,01$). In both summer and winter, an increase in swell height and therefore wave action was generally accompanied by an increase in the particulate carbon levels in the water column (Figure 2 C and D). Field et al. (1980) also recorded increases in particulate matter with increased swell height.

b) Nitrogen

Changes in particulate nitrogen levels in the water were very closely linked with fluctuations in particulate carbon levels and were also more variable in winter than in summer (Figure 2 D and E). Mean daily particulate nitrogen was 66 ($\pm$ 29) $\mu g.l^{-1}$ in summer and 79 ($\pm$ 43) $\mu g.l^{-1}$ in winter. Thus winter levels were again higher than summer levels.

c) C:N ratio

Although both carbon and nitrogen concentrations fluctuate synchronously, Figure 2 E shows that C:N ratios of the particulate load in the water column were considerably higher in winter than in summer. The winter mean was 9.90 (± 2,08) and the summer mean was 8,14 (± 1,6). Seasonal differences were significant (t-test, $p < 0,01$). Increases in swell height both in summer and winter were followed within a day by an increase in the C:N ratio (Figure 2 E and F). The mean C:N ratio of three samples of Laminaria pallida frond tips was 17,28 (± 0,01) while that of the phytoplankton/detritus from the aquarium pump filter was 7,80 (± 0,67). An increase in particulate C:N ratios is therefore likely to be a result of increased input of kelp particulate matter, rather than phytoplankton/detritus, or phytoplankton, which has a C:N ratio of between 6,40 and 7,30 (Bishop et al., 1978; Seiderer et al., 1984; Seiderer and Newell, 1985). Increased macrophyte fragmentation resulting from the strong wave action associated with predominantly northwesterly winds in winter (Figure 2 A and C), probably account for elevated winter C:N ratios.

The model of trophic relationships in the kelp bed at Oudekraal (see Wulff and Field, 1983) predicts that under continuous downwelling conditions, phytoplankton from the pelagic zone enters the kelp bed and may form up to 93% of the food available to filter feeders. In winter, onshore

winds and downwelling occur most of the time (Figure 2 A), and on the basis of the model, a high proportion of phytoplankton would therefore be expected in the particulates in the water column. A C:N ratio of 9,90 in winter implies that during continuous downwelling, a large proportion of the particulate matter is of macrophyte origin, particularly as Laminaria frond C:N ratios are only 11,70 in winter (Dieckmann, 1978). Table 2 shows that swell size is significantly correlated with particulate carbon and nitrogen levels in winter, and therefore should not be ignored in a model.

Relationship between particulate load and environmental parameters

The results of stepwise multiple correlation analysis of environmental parameters and particulate carbon and nitrogen are shown in Table 2. The summer carbon and nitrogen particulate load was most highly correlated with temperature, whereas in winter, wind and swell were the most important factors affecting carbon and nitrogen levels. Carbon was negatively correlated with wind in summer, since the prevailing offshore wind removes particulates from the area. In winter the wind/carbon correlation was positive since the onshore northwesterly winds import particulates and generate strong wave action which increases kelp frond erosion.

TABLE 2

The independent parameters influencing water column particulate carbon and nitrogen loads as determined by stepwise multiple correlation analyses ($p < 0,05$).

Dependent variable	Independent variable	Sign	Cumulative r^2
$\mu gC.l^{-1}$ Summer	Temperature	+	0,197
	Wind	-	0,288
$\mu gN.l^{-1}$ Summer	Temperature	+	0,079
$\mu gC.l^{-1}$ Winter	Wind	+	0,205
	Swell	+	0,292
$\mu gN.l^{-1}$ Winter	Wind	+	0,212
	Swell	+	0,303

Comparison of measured particulate concentration with estimates of primary production

Although the kelp bed system is not closed and there is considerable water turnover, particularly during times of strong upwelling and downwelling, a comparison can be made between the resources available for filter feeder consumption estimated from primary production, and those actually measured in the system. This is shown in Table 3. Energy balance in the various trophic levels of the kelp bed community at Oudekraal has been reviewed by Newell and Field (1983). Production of particulate organic matter by macrophytes and phytoplankton amounts to 1100 $gC.m^{-2}.y^{-1}$ while particulate nitrogen production is 150 $gN.m^{-2}.y^{-1}$. The average depth of the kelp bed is 10 m (Field et al., 1980). Thus production is 110 $mgC.l^{-1}.y^{-1}$ and 15 $mgN.l^{-1}.y^{-1}$. Although particulate carbon and nitrogen concentrations fluctuate somewhat (Figure 2 D and E) it can reasonably be assumed for the purposes of this comparison that macrophyte derived particulates and phytoplankton production enter the water column at a regular rate throughout the year. Therefore 301 $\mu gC.l^{-1}$ and 41 $\mu gN.l^{-1}$ would enter the system every day.

The present study showed that particulate carbon in the water column averaged 533 $\mu gC.l^{-1}$ in summer and 765 $\mu gC.l^{-1}$ in winter. The particulate load comprises some inorganic component. This was examined in detail by Field et al. (1980), who sampled the particulate fraction at Oudekraal over 24 days that included calm and very rough weather. From

TABLE 3

Comparison of estimates of particulate carbon and nitrogen entering the kelp bed from primary production (Newell and Field, 1983), and organic carbon and nitrogen measured in the water column. To convert carbon and nitrogen per m^{-2} to carbon and nitrogen per l^{-1}, the kelp bed is assumed to have an average depth of 10 m (Field et al., 1980), and particulates from primary production are assumed to enter the system at a uniform rate.

Primary Production	Measured particulate concentration
301 $\mu gC.l^{-1}$	380 $\mu gC.l^{-1}$
41 $\mu gN.l^{-1}$	43 $\mu gN.l^{-1}$

this study the percentage organic matter present at different swell heights may be calculated. At < 1 m and 1-2 m swell height, the organic fraction was 62% and 64% respectively of the particulate load, while at > 2 m swell height there was a decline to 53% organic matter. Thus a mean value of 63% organic matter for swell conditions of 2 m or less and 53% for > 2 m swell conditions was used. Mean daily summer particulate organic carbon and nitrogen levels were therefore 335 $\mu gC.l^{-1}$ and 41 $\mu gN.l^{-1}$, and mean winter levels were 469 $\mu gC.l^{-1}$ and 48 $\mu gN.l^{-1}$. Since the upwelling (summer) season prevails for 8 months of the year and the winter season for 4 months (Wulff and Field, 1983) the daily average over a year was 380 $\mu gC.l^{-1}$ and 43 $\mu gN.l^{-1}$. These values agree well with those calculated from production studies (Table 3).

Potential yield of particulates to consumers

Between 80 and 99 percent of particles in the water column are in the size range 5-20 µm during upwelling and downwelling (Field et al., 1980; Stuart and Klumpp, 1984), and are therefore available to the kelp bed filter feeders as food. Thus data on particulate carbon and nitrogen levels in the water column may be used to compare potential yield with the carbon and nitrogen requirements of the mussels that occur on the coast, although such energy budgets are relatively crude. Many factors such as changes in ingestion rates and gut residence times with season or particulate load, environmental factors, varying rates of protein

synthesis and rythms of digestion, absorption and excretion may play a part in regulating nutrient acquisition (for review see Bayne and Newell, 1983; Hawkins et al.,1983; Hawkins and Bayne, 1984, 1985; Widdows et al, 1984; Hawkins, 1985). Processes that occur at the benthic boundary layer may also at times modify the availability of particulate material to suspension feeders (Wildish and Kristmanson, 1984; Frechette and Bourget, 1985). The calculation of the potential yield of particulates to consumers is shown in Table 4.

There are numerous data on clearance rates, absorption efficiencies and respiration rates of the South African mussels Choromytilus meridionalis, Perna perna and Aulacomya ater (Griffiths and King, 1979a; Griffiths, 1980a,b; Stuart et al., 1982; Berry and Schleyer, 1983). Clearance and respiration rates of Mytilus galloprovincialis are not available. This species has only recently been described in South Africa, and on the west coast it has for many years been mistaken for P. perna, while P. perna is rarely found on the west coast (Grant et al., 1984; Grant and Cherry, 1985). Bayne et al (1984) describe clearance and respiration rates and the absorption efficiency of P. perna from Bloufberg Strand on the west coast. There is a good chance that these animals were in fact M. galloprovincialis, and an estimate of the clearance and respiration rates and the absorption efficiency for this species has been made on

TABLE 4

Carbon requirements of mussels compared with potential carbon absorbed from the organic particulate fraction in the water column. Carbon requirements are calculated from routine respiration rates multiplied by a factor of 2 (Bayne and Newell, 1983) using a conversion of 1 ml O_2 = 530 µgC (Hawkins and Bayne, 1985). Potential yield is obtained by multiplying clearance rate by organic carbon available. Carbon absorbed is the product of absorption efficiency and potential yield. Clearance rates and oxygen consumption values are for a temperature of 12° C.

		A. ater	C.meridionalis	M.gallo-provincialis	P.perna
Oxygen Consumption ($\mu l O_2.h^{-1}.g^{-1}$)		170	430	450	222
Carbon required ($\mu g.h^{-1}.g^{-1}$)		180	456	477	235
Clearance rate ($l.h^{-1}.g^{-1}$)		1,91	5,37	4,50	4,45
Organic Carbon available ($\mu g.l^{-1}$)	Summer	335	335	335	335
	Winter	469	469	469	469
Potential yield ($\mu g.h^{-1}.g^{-1}$)	Summer	640	1799	1507	1491
	Winter	896	2519	2110	2087
Absorption efficiency		0,50	0,40	0,40	0,61
Carbon absorbed ($\mu g.h^{-1}.g^{-1}$)	Summer	320	720	603	910
	Winter	448	1008	844	1273
% of Requirements	Summer	178%	158%	126%	387%
	Winter	249%	222%	177%	542%

the basis of those for P. perna at Blouberg Strand (see Bayne et al., 1984).

Values for oxygen consumption are for routine respiration rates at 12°C and clearance rates for all four species are for cell densities of approximately 10 x 10^6 cells.l^{-1} at the same temperature. Clearance and respiration rates of P.perna (Berry and Schleyer, 1983) were corrected from 20°C to 12°C using a Q_{10} of 2,36 calculated by Miller (quoted by Berry and Schleyer, 1983) from measurements of the rate of oxygen consumption by P. perna. The assumption is made that the same temperature coefficient applies to clearance rate (see also Bayne et al., 1984). Carbon requirements have been estimated from routine respiration rates multiplied by a factor of 2 to allow for growth and reproduction in field populations (Bayne and Newell, 1983) and a respiratory quotient of 1 ml O_2 = 530 µgC (Hawkins and Bayne, 1985). Potential yield is obtained from the product of the concentration of the resource, which appears to be always available, and the clearance rate (See Table 4).

The absorbed ration has been calculated from literature estimates of absorption efficiencies. Stuart et al. (1982) have shown that kelp debris is absorbed with an efficiency of 0,50 by the mussel A. ater. When feeding on natural detritus, C. meridionalis and P. perna had absorption efficiencies of 0,40 and 0,61 respectively (Griffiths, 1980b; Berry and Schleyer, 1983) while an absorption efficiency of 0,40 was

used for M. galloprovincialis (see Bayne et al., 1984 for P. perna at Blouberg Strand). It can be seen from Table 4 that the carbon demands of all four mussel species could easily be met in summer and winter by the organic particulate component in the water columm, despite summer upwelling events which reduce chlorophyll concentrations to low levels (Field et al., 1980; Carter, 1982; Brown, 1984; Brown and Field, 1986). It is of interest that between 127% and 249% of the carbon requirements of C. meridionalis, M. galloprovincialis and A. ater, which occur mainly on the west coast, can be met by the kelp bed particulate organic fraction, whereas over 380% of the carbon requirements of P. perna, which occurs mainly on the south and east coasts, are met. This is a result of the high absorption efficiencies and relatively low metabolic rates of P. perna (Berry and Schleyer, 1983), and is probably an adaptation to the low organic content of the south and east coast seston (Griffiths, 1980b; Berry and Schleyer, 1983; Bayne et al., 1984) compared with the west coast levels (Bayne et al., 1984; Stuart and Klumpp, 1984).

Little is known about the nitrogen requirements of South African mussels. Nitrogen requirements of Mytilus edulis vary with season, growth and reproductive condition (Bayne and Widdows, 1978; Hawkins, 1985; Hawkins and Bayne, 1985), and caution must be exercised when considering nitrogen requirements that have not been experimentally determined. However an estimate of the nitrogen requirements of these

animals can be obtained from the carbon requirements and tissue C:N ratios and is shown in Table 5. The C:N ratio of M. edulis flesh is 4,74 (Hawkins, 1983) and this was used for all four mussel species. It can be seen from Table 5 that in summer and winter, nitrogen from the particulate resource could satisfy the requirements of A. ater and P. perna. However, particulate nitrogen resources are limiting for C. meridionalis in summer and for M. galloprovincialis in summer and winter. The importance of nitrogen-rich bacteria in mussel nutrition is not clear. Bacteria in the kelp bed water column contribute a total of 99 $gN.m^{-2}.y^{-1}$ (Newell and Field, 1983). Muir et al. (1986) have shown that bacteria can be filtered from the water column by C. meridionalis. However, Stuart and Klumpp (1984) and Lucas et al. (1987) showed that bacteria cannot be filtered efficiently by mussels. The crystalline styles of both C. meridionalis and M. galloprovincialis contain enzymes capable of lysing water column bacteria (Seiderer et al., 1984; Muir et al., 1986; Seiderer, pers. comm.), and bacteria may therefore assist to a limited extent in making up the nitrogen deficit of these two species. It would be of interest to determine whether A. ater and P. perna can produce bacteriolytic enzymes.

Energy balances for various kelp bed filter feeders and for the kelp bed as a whole have been described before (Newell et al., 1982; Stuart, 1982; Newell and Field, 1983; Klumpp, 1984; Stuart and Klumpp, 1984; Seiderer and Newell, 1985). In all cases the animals described have been shown to maintain a

TABLE 5

Nitrogen requirements of mussels compared with potential nitrogen absorbed from the organic particulate fraction in the water column. Nitrogen requirements are estimated from the carbon requirements shown in Table 3 and a C:N ratio of 4,74 (Hawkins,1983) . Potential yield is obtained by multiplying clearance rate by organic nitrogen available. Nitrogen absorbed is the product of absorption efficiency and potential yield. Clearance rates are for a temperature of 12° C.

		A.ater	C. meridionalis	M.gallo-provincialis	P.perna
Nitrogen required ($\mu g.h^{-1}.g^{-1}$)		38	96	101	50
Clearance rate ($l.h^{-1}.g^{-1}$)		1,91	5,37	4,50	4,45
Organic Nitrogen available ($\mu g.l^{-1}$)	Summer	41	41	41	41
	Winter	48	48	48	48
Potential Yield ($\mu g.h^{-1}.g^{-1}$)	Summer	78	220	185	182
	Winter	92	258	216	214
Absorption efficiency		0,50	0,40	0,40	0,61
Nitrogen absorbed ($\mu g.h^{-1}.g^{-1}$)	Summer	39	88	74	111
	Winter	46	103	86	131
% of Requirements	Summer	103%	92%	73%	222%
	Winter	121%	107%	85%	262%

positive energy balance using the particulate resources available. However, a point that has not been stressed is the relevance of unchanged absorption efficiencies of many of the kelp bed filter feeders when subjected to high natural particulate loads (Griffiths, 1980b; Stuart 1982; Klumpp, 1984). In a system as variable as the kelp bed it is necessary to maintain high absorption efficiencies during periods of high particulate load, in order to maintain a positive energy balance. Kelp bed filter feeder requirements are 1297 $gC.m^{-2}.y^{-1}$ and 160 $gN.m^{-2}.y^{-1}$ (Newell and Field,1983). On a daily basis this amounts to 3,55 $gC.m^{-2}.d^{-1}$ and 0,44 $gN.m^{-2}.d^{-1}$. Since the average depth of the kelp bed is 10 m (Field et al., 1980), this represents 355 $\mu gC.l^{-1}.d^{-1}$ and 44 $\mu gN.l^{-1}.d^{-1}$. If the organic fraction comprises approximately 60% of the particulate load then filter feeders require a daily mean of 592 $\mu gC.l^{-1}$ and 73 $\mu gN.l^{-1}$ to maintain a positive energy balance. This is shown as the limiting line in Figure 2 D and E. Assuming that consumers are feeding on water of mean particle concentration (see Widdows et al., 1979; Field et al, 1980), it can be seen that periodically, particularly in summer, the filter feeder carbon and nitrogen requirements may be undersupplied. Therefore, in order acquire sufficient reserves to meet their energy requirements in times of undersupply, these organisms must be able to utilise all the available food when their requirements are oversupplied, and this requires the maintenance of high absorption efficiencies at high particulate loads. Even at the highest particulate loads

recorded in the field (1910 $\mu gC.l^{-1}$) mussel absorption efficiencies are not reduced (Griffiths, 1980b; Stuart et al., 1982; Berry and Schleyer, 1983).

CONCLUSIONS

The physical factors affecting particulate carbon and nitrogen concentrations in the kelp bed have been described. Summer upwelling events do not restrict the food available to mussels that occur on the South African coastline, although nitrogen resources may be limiting for C. meridionalis and M. galloprovincialis. Winter particulate concentrations are higher than summer levels and comprise a greater proportion of kelp detritus. Food resources measured in the water column closely match those predicted from primary production studies. Although winter particulates appear to be comprised largely of material originating from macrophytes in the system, it is not clear what part phytoplankton plays in the nutrition of resident filter feeders, and seasonal changes may occur in the composition of the food available. Using High Performance Liquid Chromatography, the pigment matrix in the water column is investigated in the next chapter, to determine qualitative changes that may occur in particulate material in the water column.

CHAPTER II

THE QUALITATIVE NATURE OF THE PARTICULATE RESOURCE

INTRODUCTION

On the west coast of South Africa frequent pulses of upwelling, generated by strong south-easterly winds result in the rapid escalation of nutrient concentrations in the inshore and offshore regions. Once upwelling relaxes or downwelling occurs, phytoplankton production increases very rapidly in response to the high nutrient levels (Andrews and Hutchings, 1980; Field et al., 1980; Carter, 1982; Brown and Field, 1986). Phytoplankton thus represents a potential food source for filter feeders in the inshore region. Although high C:N ratios indicate that in winter much of the particulate material in the kelp beds derives from macrophytes (Chapter I), phytoplankton contributes approximately 50% of the primary production in the inshore area (Newell et al., 1982), and it is not clear how important this component is in the diet of filter feeders utilizing the particulate resource. Phytoplankton cells are generally enclosed in a cellulose or silica based cell wall, but contain considerable protein, carbohydrate and lipid reserves (Raymont, 1980; Barlow, 1982). Kelp derived detrital material is likely to consist mainly of structural polysaccharides and some laminarin storage products. Thus the food value of the two particulate resources, the ease with which such foods are digested and the enzymes neccessary for such digestion will be different. By following changes in the chlorophyll-like pigments of the particulate material in the water column in

relation to environmental variables, factors affecting the growth and status of the phytoplankton population in the kelp bed system can be determined. Using chlorophyll a : carbon ratios, a good idea of the relative contribution of macroalgal detritus and phytoplankton particulates to the diet of the filter feeders in the area may be obtained.

Chlorophyll concentrations have been used for many years as a determinant of phytoplankton biomass in oceans around the world. Lorenzen and Jeffrey (1980) describe the development of methods for estimating phytoplankton chlorophylls, using spectrophotometric and fluorescence techniques. Schwartz and von Elbe (1982) review the systems available for the analysis of plant pigments. Briefly, Richards and Thompson (1952) described some of the first trichromatic equations which were later replaced by improved versions (Scor-Unesco, 1966; Strickland and Parsons, 1972). Trichromatic equations based on the most recently determined chlorophyll extinction coefficients are given by Jeffrey and Humphrey (1975). The introduction of fluorometric techniques greatly improved the sensitivity of chlorophyll measurements (Yentsch and Menzel 1963; Holm-Hansen et al., 1965). However, both these methods suffer from the fact that chlorophyll degradation products are not distinguished from active chlorophyll and as a result, phytoplankton biomass may be considerably overestimated. These degradation products may constitute a significant fraction of the green pigments, particularly in

senescent phytoplankton or phytoplankton/detrital assemblages (Mantoura and Llewellyn, 1983).

Lorenzen (1967) introduced an acidification procedure which alters the spectral properties of chlorophyll a by converting it to phaeophytin a, but does not alter the spectral properties of breakdown products such as phaeophytin and phaeophorbide. The change in absorbance before and after acidification could thus be related to the amount of chlorophyll a originally present. Although this step constituted an improvement, it did not resolve the main difficulty inherent in the spectrophotometric and fluorometric determination of chlorophyll, namely the inability to separate the matrix of pigments from their degradation products, present in most natural phytoplankton populations. Other chlorophyll degradation products such as chlorophyllide a, which has a similar absorbance maximum and only slightly lower molar extinction to chlorophyll a, may constitute a significant proportion of the chlorophyll fraction that is converted to "phaeopigments". Also, carotenoid pigments, which may be better indicators of algal biomass (Shimura and Fujita, 1975), are not determined by this method. Carotenoids such as fucoxanthin, a major pigment in diatom populations (Raymont, 1980), undergo spectral changes on acidification, which interfere with the determination of chlorophyll a and phaeopigments (Rieman, 1978, 1982).

Separation of pigments by various means overcomes the problems of their mutual interference during analysis. Paper chromatography (Holden, 1962; Jensen, 1978), column (Strain et al., 1968, 1971) and thin layer chromatography (Jeffrey, 1968, 1974, 1981; Garside and Riley, 1969; Daley et al., 1973; Shiraki et al., 1978;) have been used for this purpose, but the techniques are often slow and insensitive and are not compatible with large sample numbers and photolabile pigments. High Performance Liquid Chromatography (HPLC) has recently been used to overcome these limitations (Jacobsen, 1978, 1982; Brown et al., 1981; Wright and Shearer, 1984; Mantoura and Llewellyn, 1983; Monteiro, 1986; Monteiro et al., 1986), and reverse phase conditions are preferred to normal phase because the polar stationary phases of the latter promote pigment degradation (Braumann and Grimme, 1981). Comparisons of the results of HPLC, spectrophotmetric and fluorometric determinations of chlorophyll a in phytoplankton populations have generally indicated that chlorophyll a concentrations are seriously over-estimated by the last two methods (Jacobsen, 1978, 1982; Mantoura and Llewellyn, 1983).

Although HPLC separation of algal pigments offers a method for accurate quantification of the various elements of the water column pigment matrix, as yet few studies have made use of this method to analyse fluctuations in various components of the matrix as an indicator of physiological and structural changes in the water column algal population. The amount of

chlorophyllide, phaephorbide and phaeophytin present in the water column can provide valuable information on the status of the phytoplankton population and the grazing pressure on it (Jeffrey, 1974; Gieskes et al., 1978; Hallegraeff, 1981; Hawkins et al., 1986). Analysis by HPLC of the pigments present in the water column during cyclical upwelling and downwelling events can provide insight into the nature of changes occurring in the phytoplankton population.

The following work was carried out with three objectives:

1. To compare HPLC and spectrophotometric estimates of chlorophyll a concentrations in order to assess whether previous values for chloropyll a concentrations in the kelp bed area are likely to have overestimated the importance of phytoplankton as a food source.
2. To examine changes in the water column pigment matrix with season and environmental variables in order to determine qualitative changes in the phytoplankton food source.
3. To establish chlorophyll:carbon ratios for the near-shore region in order to assess the relative importance of living phytoplankton and detrital material as food sources for filter feeders in the area.

MATERIALS AND METHODS

Sampling procedure: Chlorophyll a

Water samples were collected daily at 0800 h from the shore at Oudekraal, on the west coast of the Cape Peninsula, for 52 days in November/December 1984, which is the principal summer upwelling season (Andrews and Hutchings, 1980), and for 31 days in June/July 1985 (winter). The sampling procedure is described in Chapter I. For chlorophyll analyses, between 100 ml and 900 ml, depending on particulate load, was filtered by gentle hand pump filtration, in subdued light, first through a 200 µm mesh and then through Whatman GF/F filters. Four samples were taken daily, two being for analysis by HPLC and two for analysis by spectrophotometry. Owing to constraints imposed by expense and lack of machine time, one sample was analysed for algal pigments by HPLC and the same sample, as well as two others, were analysed for chlorophyll *a* and phaeopigments by spectrophotometry. No magnesium carbonate wash was added to filters because it can bind chlorophyllides and phaeophytins (Daley *et al.*, 1973). After filtration, filter papers were folded, wrapped in aluminium foil and stored in liquid nitrogen until analysed.

Extraction of pigments

Solvents for extraction and chromatography were all of Analar grade. Pigments were extracted using 90% acetone. Although Holm-Hansen (1978) suggests that methanol has a better

extraction efficiency for HPLC and spectrophotometric determinations of chlorophyll pigments, Mantoura and Llewellyn (1983) showed that methanol can enhance chlorophyllase activity, which rapidly converts chlorophyll a to chlorophyllide. Filters were ground in 2,5 ml 90% acetone for one minute with a motorized tissue grinder. The slurry was transferred to a centrifuge tube and centrifuged at 12000 x g for 60 secs. Chlorophyll a is converted to chlorophyllide a very rapidly, especially when samples contain a large proportion of diatoms (Monteiro et al., 1986), so samples were extracted and analysed in the shortest possible time (approximately 12 minutes).

HPLC analysis

Exactly 300 µl of the acetone extract was removed with a Hamilton syringe and mixed with 100 µl of an ion-pairing reagent prepared from 1,5 g tetrabutyl-ammonium acetate and 7,7 g of ammonium acetate made up to 100 ml with water (Mantoura and Llewellyn, 1983). The chromatographic separations were carried out using a Beckman HPLC system comprising two model 112 pumps and a 340 organiser, coupled to a Drew Scientific Chromatography Interface linked to an Apple IIC terminal for both gradient control and peak integration. A Beckman model 165 variable wavelength detector was used with a wavelength setting of 440 nm. A 100 µl injection loop was used and separations were carried out using a 7 cm Altex XL ODS reverse phase column with 3 µm packing. This column allowed the shortening of analysis time

from 18 to 8,5 minutes without loss of resolution, thus making the method suitable for large numbers of samples (Monteiro et al., 1986). The composition of the two mobile phase solvents was as described by Mantoura and Llewellyn (1983), but the gradient profile was changed to suit the shorter column. Figure 3 shows the gradient profile used.

Peaks were identified by comparison with standards processed by Pedro Monteiro, and calibration for the quantification of chlorophyll a and chlorophyllide a was carried out using a chlorophyll a standard (Sigma Chemical Company, Product code: C-6144) made up to known volume in 90% acetone and stored at -20^{0}C in the dark (Mantoura and Llewellyn, 1983). The corresponding concentrations of breakdown products, xanthophylls and carotenoids were determined using the ratios of the molar extinction coefficients of chlorophyll a and the known component of τ = 440 nm (Monteiro et al., 1986; Monteiro, pers. comm.). Chlorophyll b, chlorophyll c, lutein and phaephorbide a were not quantitatively analysed but simply described as present or absent in the chromatograms.

Spectrophotometric analysis

Exactly 1,6 ml of each extract analysed by HPLC was transferred to a 1 cm cuvette and read at 750, 665, 664, 647 and 630 nm in a Beckman model 25 spectrophotometer. Thereafter, 25 µl of 0,2 M HCl was added to achieve a final concentration of 3×10^{-3} M HCl, so reducing spectral changes caused by acidification (Holm-Hansen, 1978; Rieman, 1978).

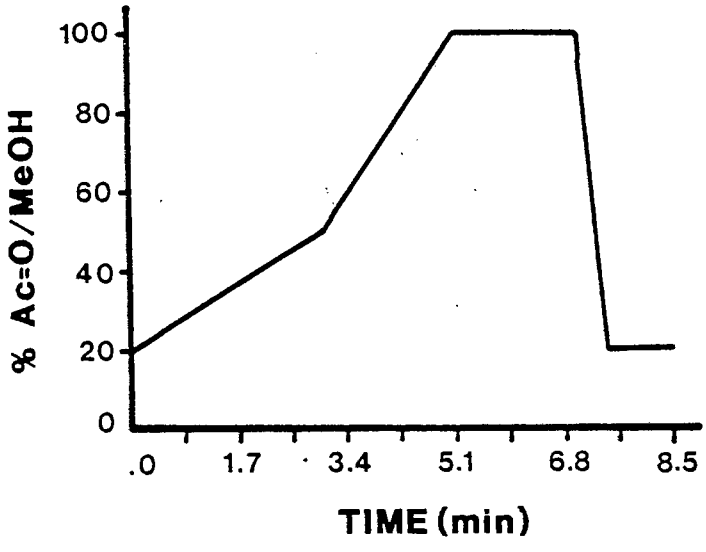


Figure 3. Solvent gradient used in HPLC analysis of pigments of the kelp bed particulate fraction.

The cuvette was inverted twice and allowed to stand for one minute before readings were taken at 750 and 665 nm. Two other samples from each days sampling were processed in a similar manner.

Calculation of pigment concentrations

Chlorophyll a and phaeophytin a concentrations of the samples analysed by HPLC were compared with concentrations calculated spectrophotometrically using the Jeffrey and Humphrey (1975) trichromatic equations (chlorophyll a only) and the Lorenzen (1967) equations for chlorophyll a and phaeopigments, using a paired t-test (Statpro, Wadsworths). The mean chlorophyll a concentrations of the three daily samples analysed spectrophotometrically and calculated by the Jeffrey and Humphrey (1975) and Lorenzen (1967) methods were also compared with chlorophyll a values calculated from HPLC analyses (paired t-test, Statpro).

Qualitative pigment standards by HPLC

In order to identify the origin of some of the pigments present in particulate matter from the water column, extracts from a number of common algal and phytoplankton types were analysed by HPLC. These were not quantitatively assessed, but used only to identify the major pigments associated with each algal group. The macrophytes were freshly collected on the day of analysis and the phytoplankton species obtained from cultures used for feeding experiments. Discs of macrophyte

fronds were punched out and homogenized in 90% acetone, while phytoplankton were filtered through GF/F filters. All extracts were then treated as described above and the peaks were identified from standards processed by Pedro Monteiro. Algae treated in this manner are shown in Table 6.

RESULTS AND DISCUSSION

Chromatograms of common algae

Figures 4 and 5 show chromatograms of various algal types, many of which occur in the kelp bed system. Pigments were completely separated by the HPLC system used. The kelp bed macrophytes Ecklonia maxima and Laminaria pallida and the diatoms Chaetoceros gracilis and Phaeodactylum tricornutum showed similar pigment profiles. Major pigments were chlorophyll c, fucoxanthin and chlorophyll a, and minor pigments were diatoxanthin and diadionaxanthin (Figure 4). Tayloriella virgata is a common kelp epiphyte while Gigartina radula is present intertidally and below the kelp canopy. The major pigments present were fucoxanthin, lutein and chlorophyll a. However, the samples were contaminated, probably with other minute epiphytic algae, since both chromatograms show the presence of chlorophyll c, diatoxanthin and diadionaxanthin, and chlorophyll b was present in the T. virgata chromatogram (Figure 5).

TABLE 6

Algae used for qualitative pigment analysis by HPLC in order to identify the origin of some of the pigments present in particulate matter from the kelp bed.

MACROPHYTES	ALGAL GROUP
Ecklonia maxima	Phaeophyta
Laminaria pallida	Phaeophyta
Tayloriella virgata	Rhodophyta
Gigartina radula	Rhodophyta
Ulva lactuca	Chlorophyta
PHYTOPLANKTON	
Phaeodactylum tricornutum	Bacillariophyceae
Chaectoceros gracilis	Bacillariophyceae
Tetraselmis suecica	Prasinophyceae

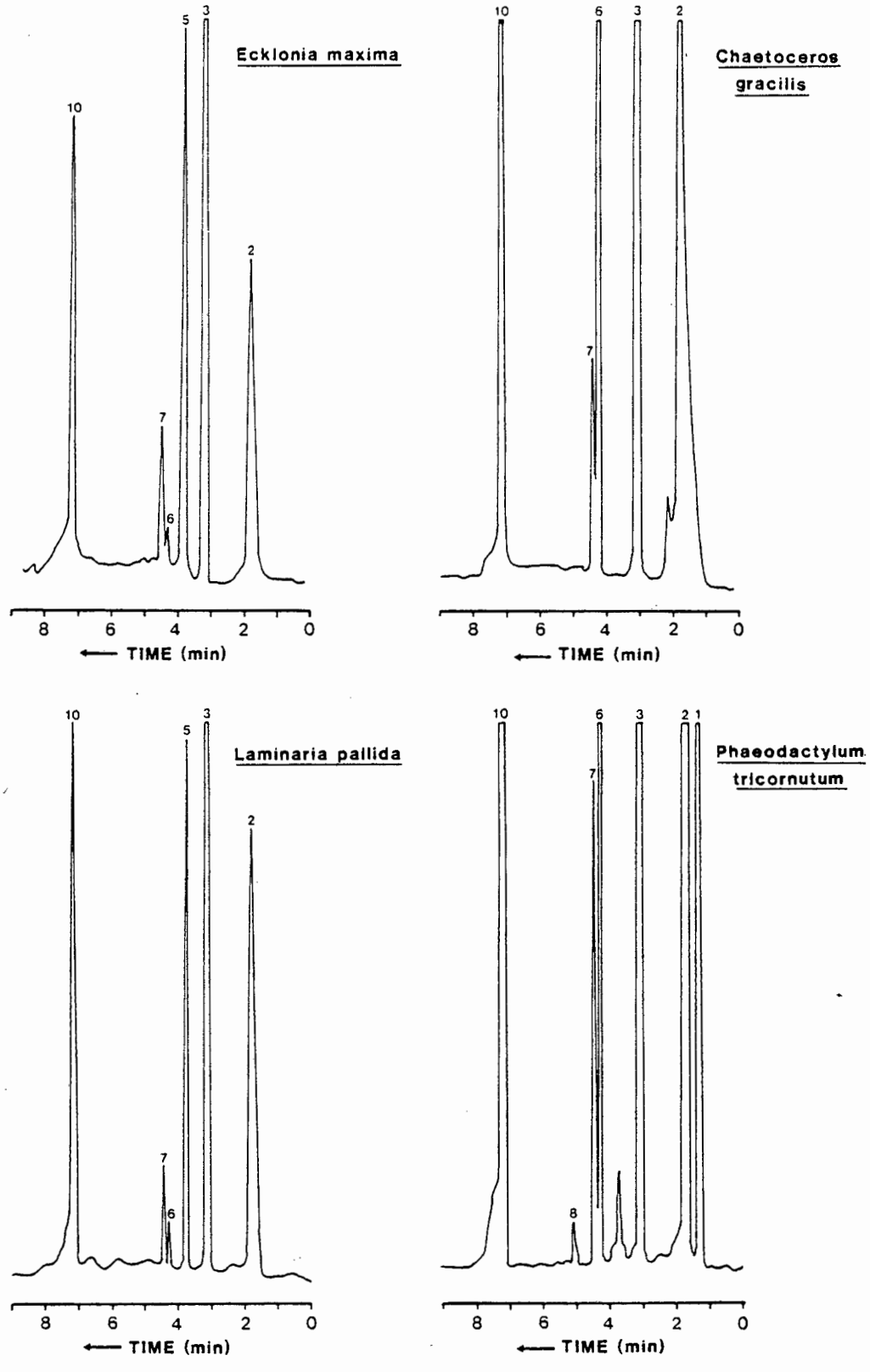


Figure 4.
HPLC chromatograms of different algal types that may occur in the kelp bed . Peak identities are:

1	chlorophyllide a	2	chlorophyll c	3	fucoxanthin
4	neoxanthin	5	violxanthin	6	diatoxanthin
7	diadionaxanthin	8	lutein	9	chlorophyll b
10	chlorophyll a				

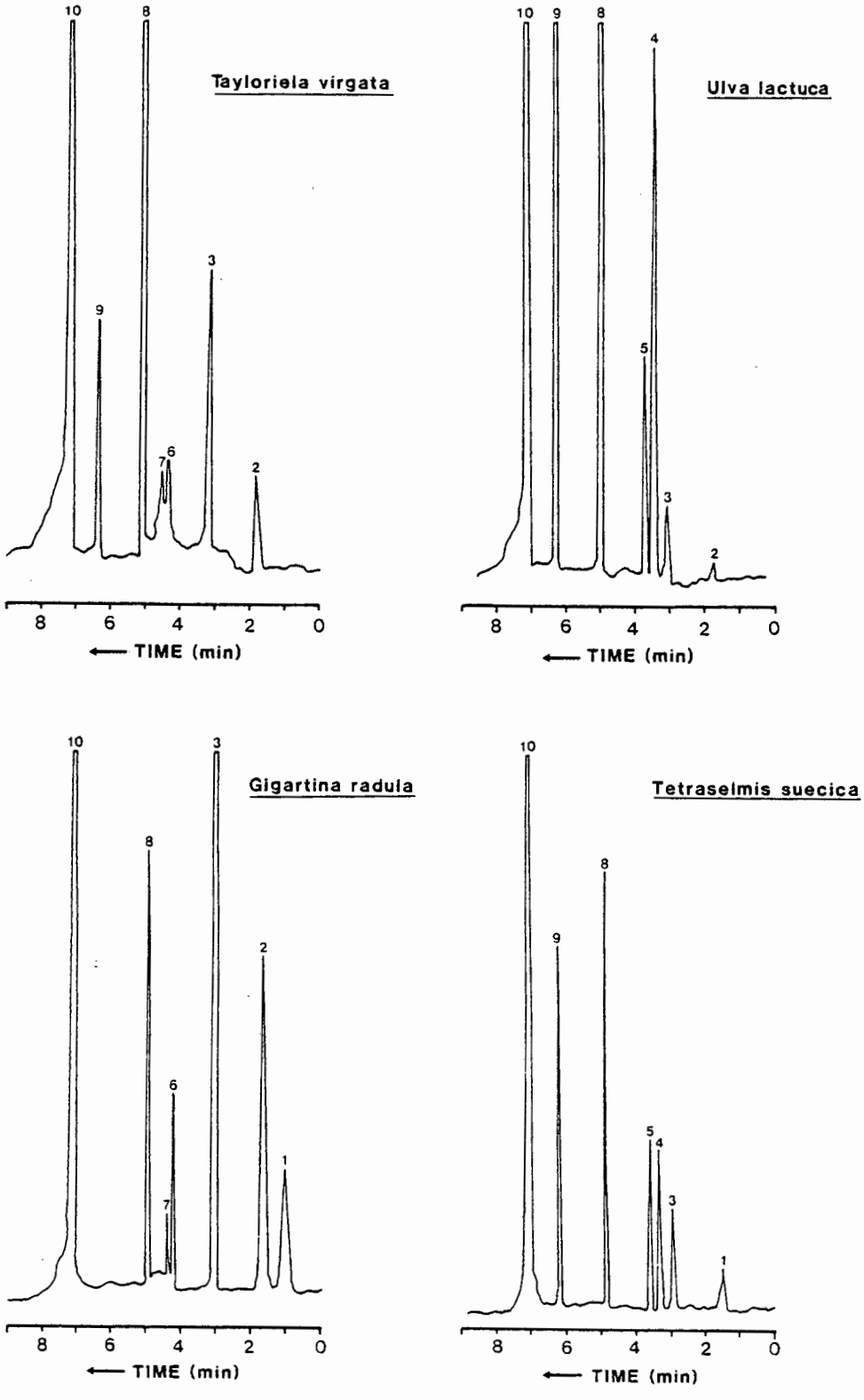


Figure 5.
HPLC chromatograms of different algal types that may occur in the kelp bed . Peak identities are:

1	chlorophyllide a	2	chlorophyll c	3	fucoxanthin
4	neoxanthin	5	violxanthin	6	diatoxanthin
7	diadionaxanthin	8	lutein	9	chlorophyll b
10	chlorophyll a				

The green algae Ulva lactuca and Tetraselmis suecica had major chlorophyll b peaks, as well as chlorophyll a and lutein. Violxanthin and neoxanthin were present as minor peaks in both samples (Figure 5). T. suecica used for HPLC analysis had been cultured for feeding experiments and microscopic examination revealed slight contamination of the culture by P. tricornutum, which explains the small chlorophyll c and fucoxanthin peaks in the chromatogram. These chromatograms show that in the kelp bed area the diatoms and canopy macrophytes are likely to be the source of much of the chlorophyll c and fucoxanthin pigments present in the particulate material of the water column (Figure 4). The presence of lutein in samples indicates that the understorey macrophytes (see Figure 5) may also contribute to the particulate fraction, since lutein is not present in E. maxima and L. pallida (Figure 4). Any chlorophyll b in particulate material will result from the presence of green algae.

Examples of HPLC analysis of water column pigments from the kelp bed area, together with peak identities are shown in Figure 6.

Comparison of HPLC and spectrophotometric methods

All three methods of estimating chlorophyll concentrations generally indicated the same trends, major increases and decreases in chlorophyll a co-inciding closely during both summer and winter, (Figures 7,8,9 and 10). Mean summer and

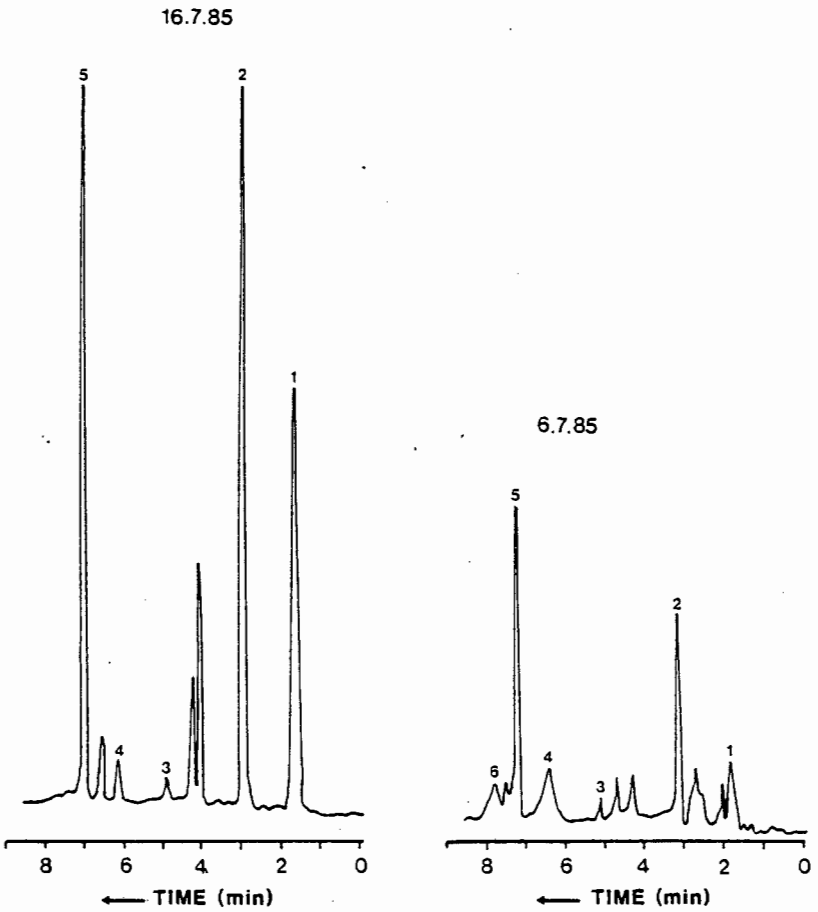


Figure 6.
Separation of pigments present in the kelp bed water column on two separate days, using a 7 cm Altex reverse phase column.
Peak identities are:

1	Chlorophyll c	2	Fucoxanthin	3	Lutein
4	Chlorophyll b	5	Chlorophyll a	6	Phaeophytin a

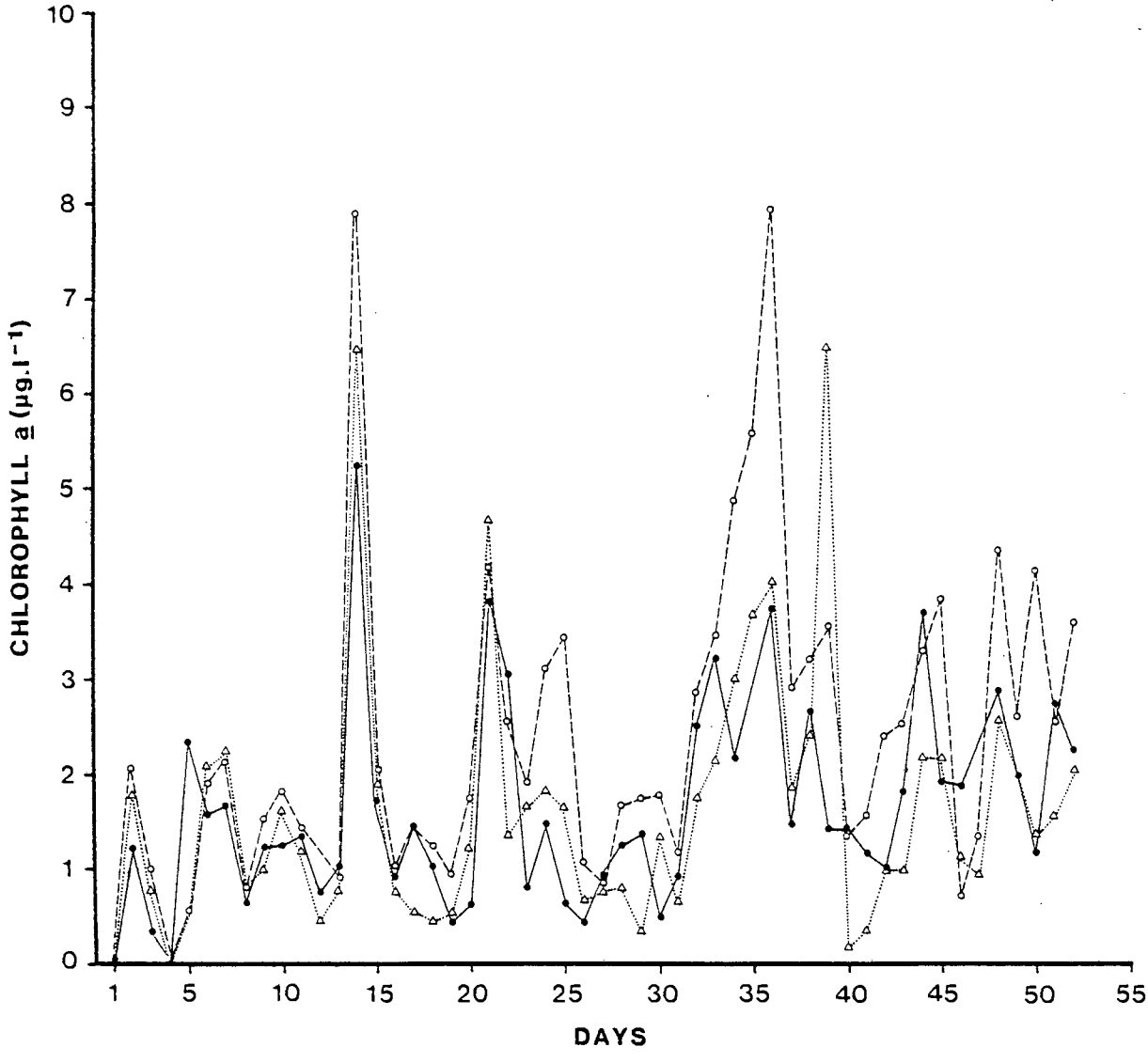


Figure 7. A comparison of summer chlorophyll a concentrations ($ug.l^{-1}$) in the kelp bed, determined by HPLC (●——●) and spectrophotometrically by the equations of Jeffrey and Humphrey (1975) (o-----o) and Lorenzen (1967) (△·····△).

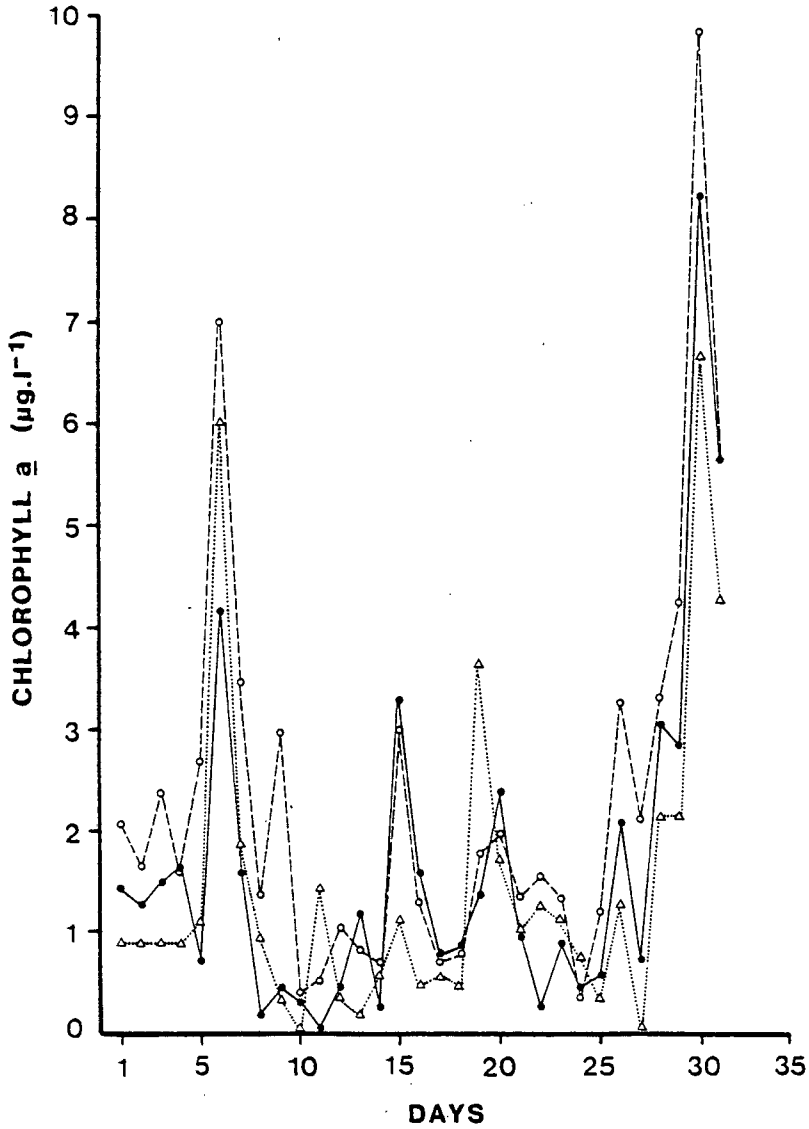


Figure 8. A comparison of winter chlorophyll a concentrations ($ug.l^{-1}$) in the kelp bed, determined by HPLC (●——●) and spectrophotometrically by the equations of Jeffrey and Humphrey (1975) (o----o) and Lorenzen (1967) (△·····△).

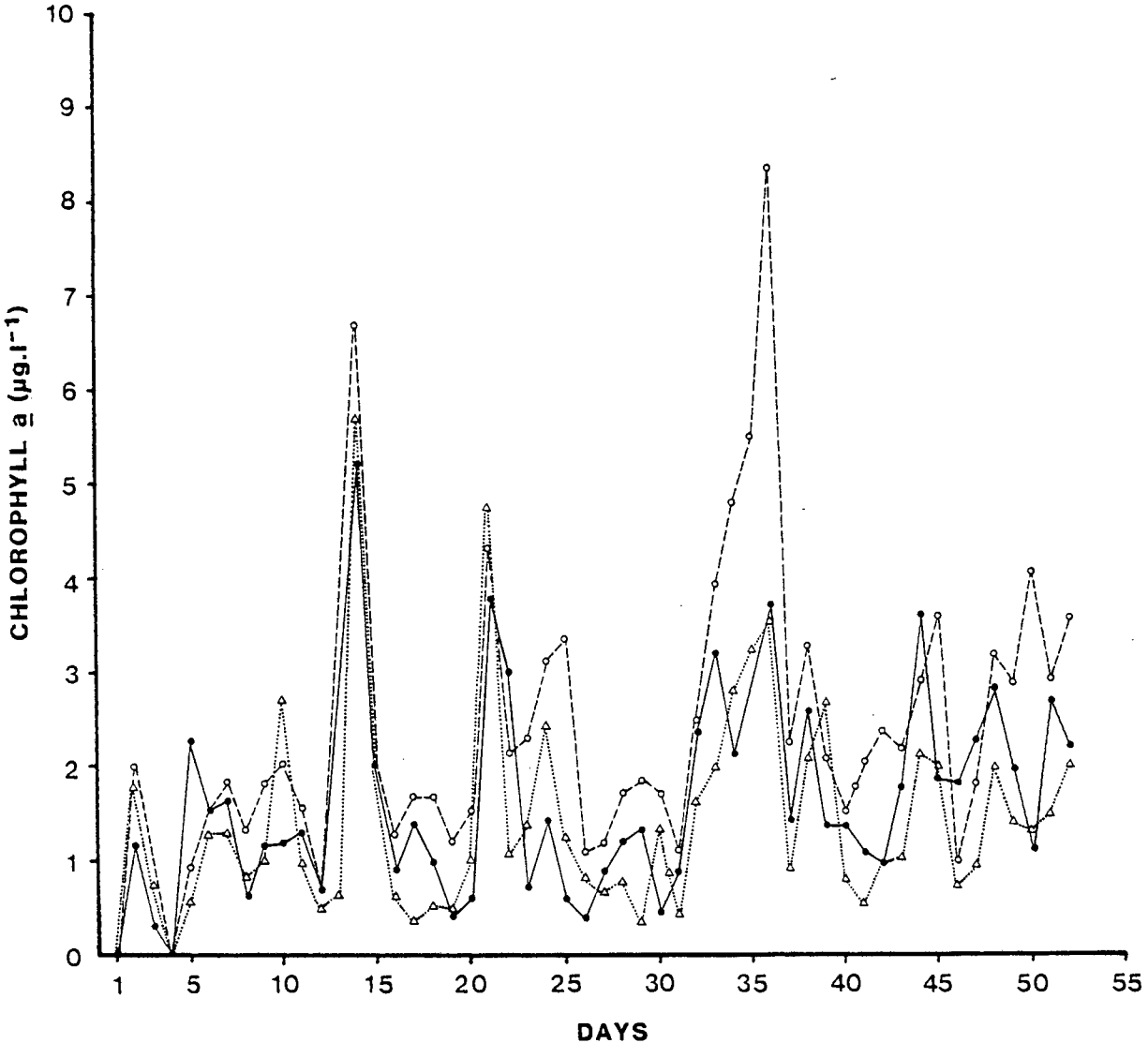


Figure 9. Summer kelp bed chlorophyll a concentrations (ug.l^{-1}) determined by HPLC (●——●) and the mean of three samples determined spectrophotometrically by the equations of Jeffrey and Humphrey (1975) (o-----o) and Lorenzen (1967) (△·····△).

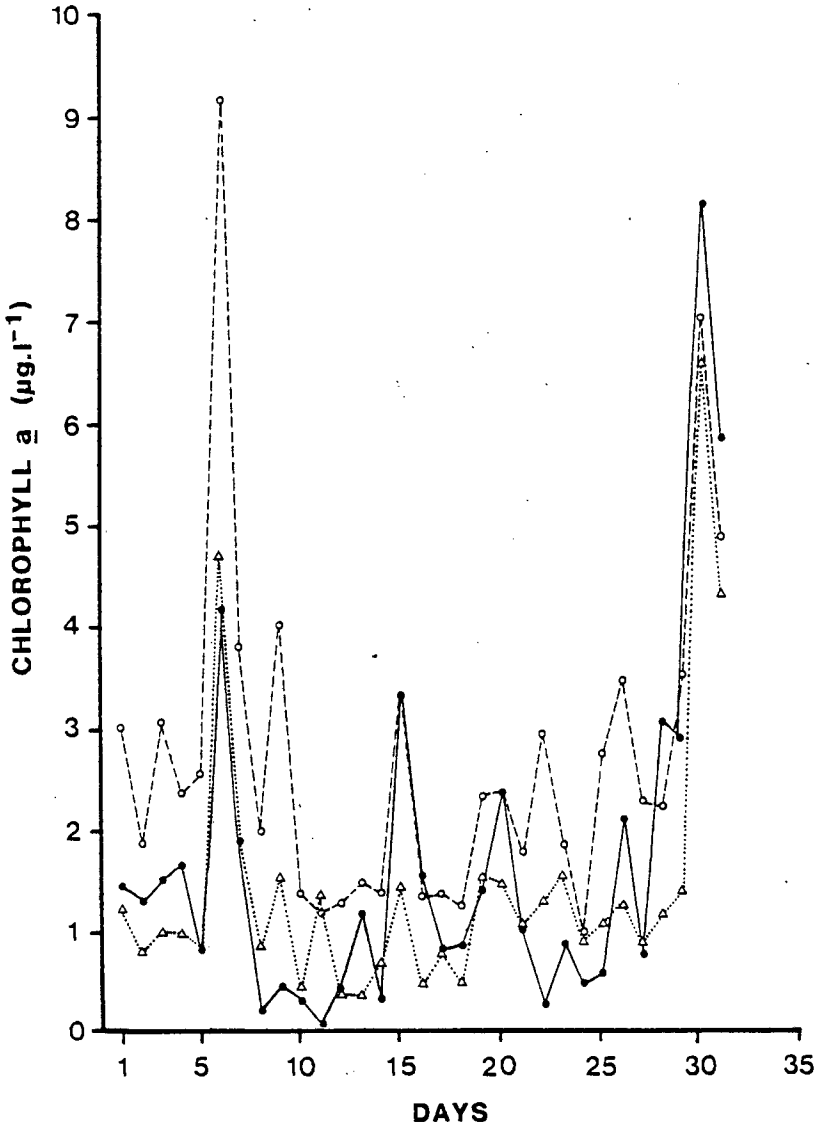


Figure 10. Winter kelp bed chlorophyll a concentrations ($ug.l^{-1}$) determined by HPLC (●——●) and the mean of three samples determined spectrophotometrically by the equations of Jeffrey and Humphrey (1975) (o----o) and Lorenzen (1967) (△·····△).

winter chlorophyll a concentrations estimated by the three methods are shown in Table 7. There were no significant differences between pairs of summer and winter values (t-test, $p > 0,01$).

Daily chlorophyll a concentrations calculated from the same sample analysed by HPLC, Jeffrey and Humphrey (1975) and the Lorenzen (1967) methods are shown in Figures 7 and 8. HPLC estimations of chlorophyll a concentrations are compared with chlorophyll a concentrations estimated from the mean of three daily samples analysed spectrophotometrically by the Jeffrey and Humphrey (1975) and Lorenzen (1967) methods in Figures 9 and 10. Chlorophyll a concentrations calculated using the Jeffrey and Humphrey (1975) trichromatic equations were generally higher than HPLC estimations, and the two methods provided significantly different estimates, both for the single sample series shown in Figures 7 and 8 and the sample mean series shown in Figures 9 and 10 (paired t-test, $p < 0,001$). No significant difference between the Lorenzen (1967) and HPLC estimates of chlorophyll a concentrations was found (paired t-test, $p > 0,05$). The correlation between HPLC and Jeffrey and Humphrey (1975) trichromatic values for chlorophyll a extracted in 90% acetone is shown in Figure 11. Estimates of chlorophyll a by the Jeffrey and Humphrey (1975) method were higher than those measured by HPLC ($y = 0,51 + 1,12x$) and the correlation coefficient was 0,83.

TABLE 7

Mean chlorophyll *a* concentrations (± S.D.) in the kelp bed water column during summer and winter, estimated by three different methods

Method	Summer chlorophyll *a* $\mu g.l^{-1}$	Winter chlorophyll *a* $\mu g.l^{-1}$
HPLC	1,64 (± 1,06)	1,78 (± 1,90)
Jeffrey and Humphrey (1975)	2,37 (± 2,82)	2,31 (± 2,05)
Lorenzen (1967)	1,63 (± 1,92)	1,47 (± 1,61)
Jeffrey and Humphrey (1975)		
Mean of 3 samples	2,39 (± 2,39)	2,67 (± 1,75)
Lorenzen (1967)		
Mean of 3 samples	1,46 (± 1,24)	1,42 (± 1,35)

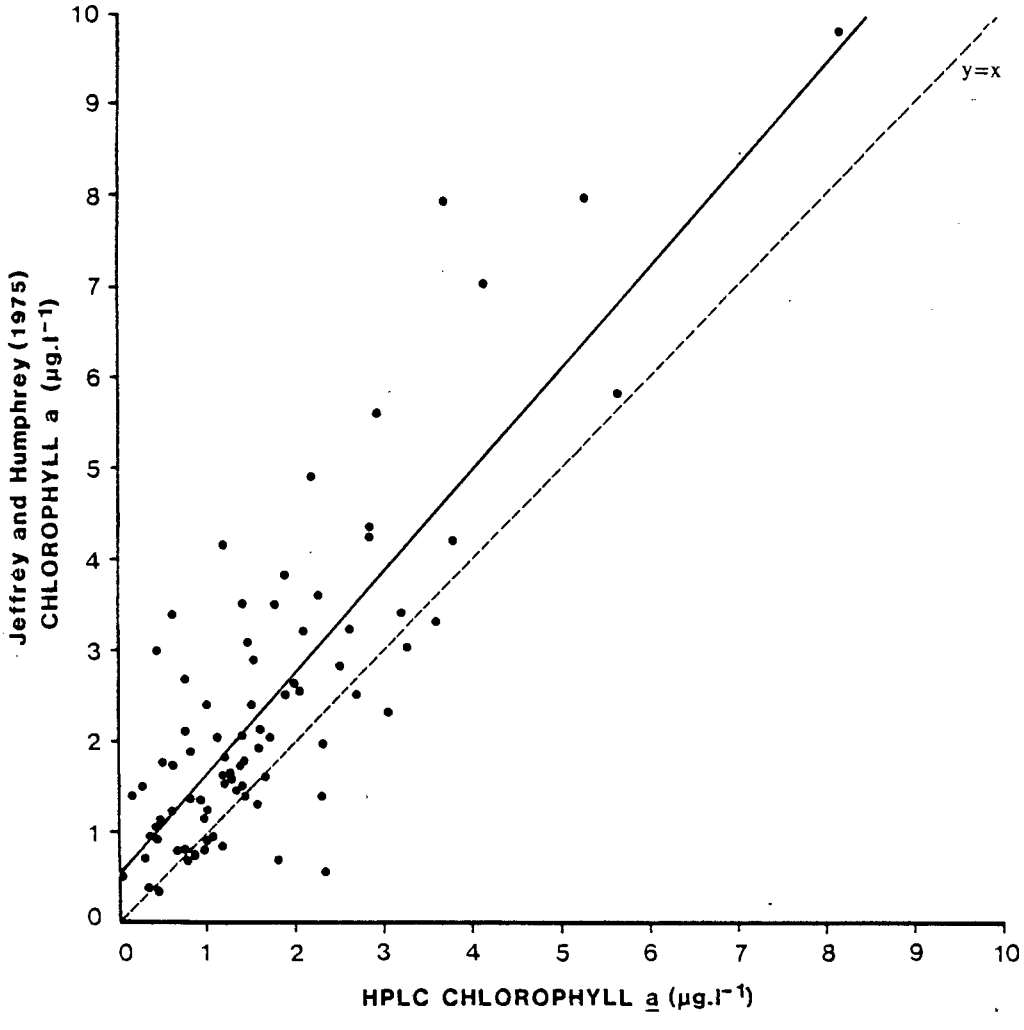


Figure 11. The relationship between chlorophyll a concentrations determined by HPLC and spectrophotometrically by the trichromatic equations of Jeffrey and Humphrey (1975). Regression equation: $y = 0,51 + 1,12x$; $r = 0,83$, $n = 83$. The line $y = x$ is also shown.

It has been shown previously that spectrophotometric methods of estimating chlorophyll a in highly productive lakes and the estuarine benthos overestimate chlorophyll a concentrations by factors of between 2 and 12,6 when compared with HPLC estimates (Jacobsen, 1978,1982; Mantoura and Llewyllyn, 1983). However there was fairly good correlation between spectrophotometric and HPLC results when samples were taken from the open sea (Mantoura and Llewellyn, 1983). In the nearshore environment at Oudekraal, chlorophyll a concentrations determined by the method of Jeffrey and Humphrey (1975) ranged between 0,2 and 9,8 times the corresponding concentrations determined by HPLC (Figures 7 and 8), with 65% of the values being between one and three times the HPLC values.

The discrepancy between trichromatic and HPLC estimations of chlorophyll a will be greatest when there is a build up of pigment breakdown products such as chlorophyllides and phaeophorbides. Chlorophyllase activity is particularly vigorous in diatoms (Barret and Jeffrey, 1964). The reduced presence of fucoxanthin and chlorophyll c in the summer phytoplankton population (see pp. 77 and 79) indicated that diatoms were not well represented in the phytoplankton blooms. Their absence together with the associated chlorophyllase activity, would contribute to reducing the discrepancy between the two methods. In contrast, Mantoura and Llewellyn (1983) analysed sediment samples, in which diatoms are generally the dominant photoautotrophs, and thus

chlorophyllase activity would be enhanced. Moreover, because of the wind regime, there is considerable movement of water into and out of the kelp bed system (Field et al., 1980). Thus, although phytoplankton blooms occurred at regular intervals (Figures 7 and 8), the phytoplankton together with their associated chlorophyll precursors and degradation products may remain in the system for only a brief time, thus reducing any build-up of degradation products.

Comparisons of previous estimates of kelp bed chlorophyll a with HPLC estimates

The chlorophyll a concentrations and fluctuations recorded using HPLC are similar to the summer values recorded by Field et al. (1980) in the kelp bed area, using the Scor-Unesco (1966) trichromatic equations. The chlorophyll a values of Field et al. (1980) vary from hardly detectable to peak values of 10 µg Chl $a.l^{-1}$. However some other chlorophyll a values for the kelp bed area are considerably higher than those recorded by HPLC, and these are shown in Table 8. Chlorophyll a concentrations such as those shown in Table 8 were never measured by HPLC in the present study (Figures 7 and 8). Neither the Scor-Unesco method used by Brown (1984) and Brown and Field (1986), nor the chlorophyll calibration curve used by Barlow (1982), corrects for degradation products. In the phytoplankton blooms in mature and aged upwelled water, considerable amounts of degradation products such as chlorophyllide, phaeophorbide and phaeophytin are probably present, which will result in an overestimation of

TABLE 8

Previous estimates of chlorophyll *a* concentrations under different conditions in the kelp bed area at Oudekraal.

Chlorophyll *a* ccn. $\mu g.l^{-1}$	Conditions	Method	Source
0-24	Upwelling/ downwelling	SCOR-UNESCO (1966)	Brown, 1984
11-18	Downwelling	SCOR-UNESCO (1966)	Brown and Field (1986)
0-20	Mature upwelled water	Chlorophyll calibration curve	Barlow, (1982)
5-30	Aged upwelled water	Chlorophyll calibration curve	Barlow, (1982)

chlorophyll a concentrations (Jacobsen 1978, Jacobsen 1982; Mantoura and Llewellyn, 1983; Monteiro et al., 1986). Shumann and Lorenzen (1975) showed that grazing by herbivores results in degradation products, and actively growing populations of photoautotrophs contain chlorophyllides and other chlorophyll precursors. Whitney and Darley (1979) found that pigment extracts of log phase growth cultures of diatoms contain about 10% chlorophyllide and phaeophorbide, while Jeffrey (1974) has shown the presence of significant quantities of chlorophyllides and phaeophorbides in phytoplankton blooms off Australia. Thus it appears that previous studies (Barlow, 1982; Brown, 1984; Brown and Field, 1986) have somewhat overestimated chlorophyll a concentrations and hence phytoplankton biomass in the water column at Oudekraal.

Chlorophyll a, Nitrates and the Environment

Environmental conditions prevailing during the time series, together with chlorophyll a concentrations determined by HPLC, and nitrate concentrations taken from Muir (1986) are shown in Figures 12 and 13. The relationship between the wind regime and temperature has been described in Chapter I. In summer pulses of strong southeasterly winds caused upwelling of cold, nitrate rich water into the kelp bed system (Figure 12 A, B and D). Southeasterly winds cause an offshore movement of surface waters (Andrews and Hutchings, 1980; Field et al. 1980), and during upwelling the chlorophyll a concentration in the inshore waters was low ($\pm$ 1 $\mu g.l^{-1}$). However, when southeasterly winds relaxed or changed to

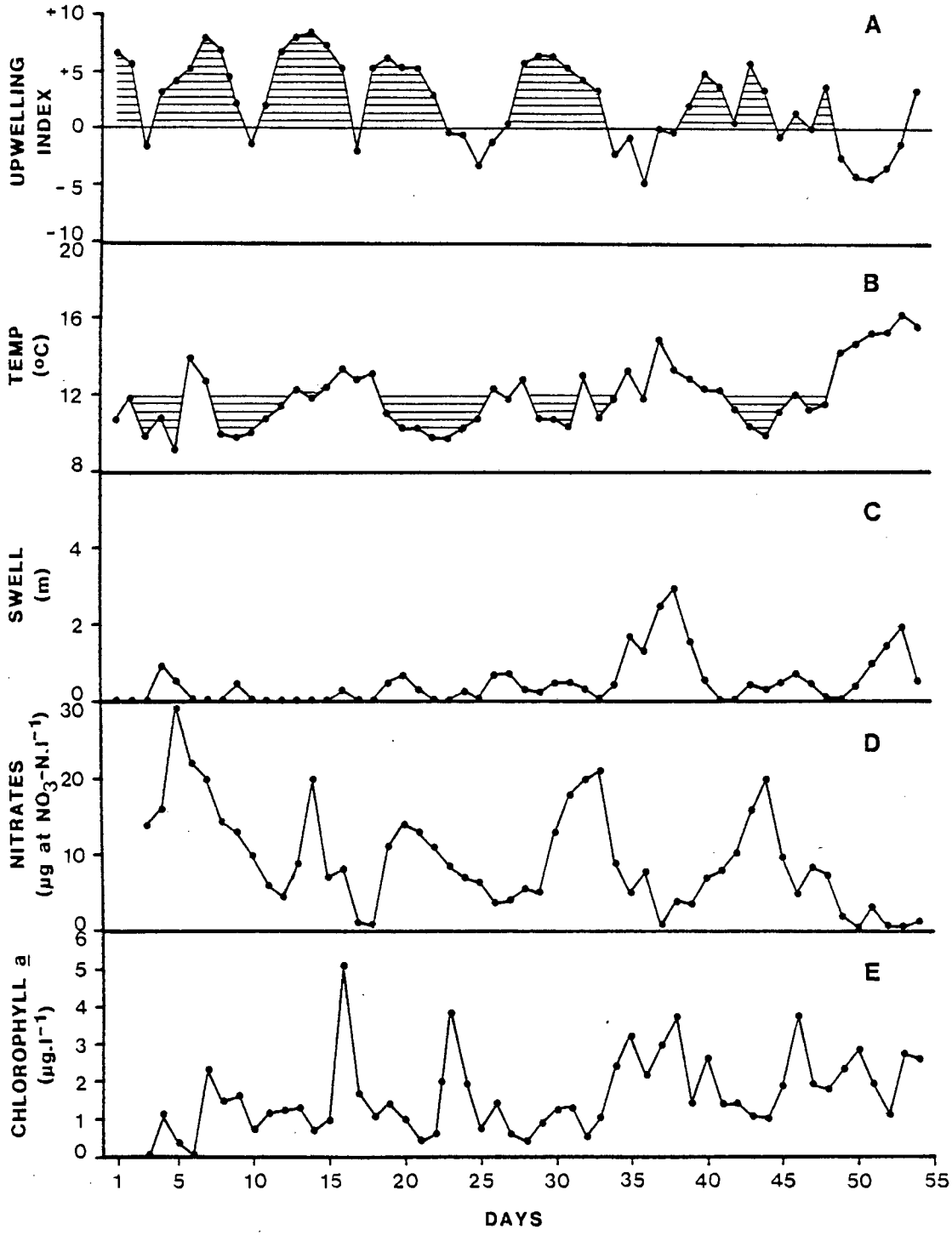


Figure 12. Variations in physical factors, nitrate and chlorophyll a concentrations in the kelp bed at Oudekraal during summer. Nitrate concentrations are from Muir (1986).

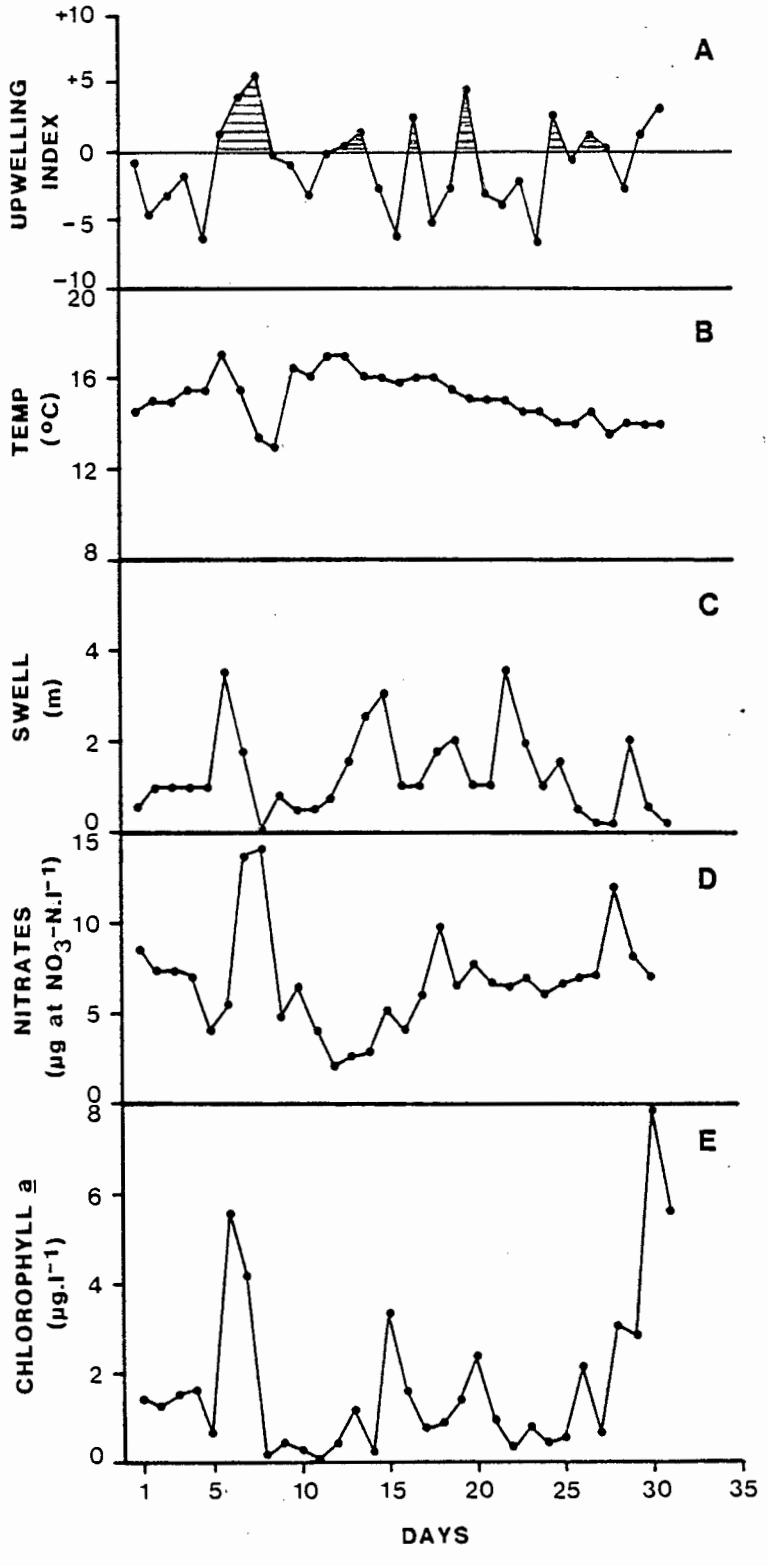


Figure 13. Variations in physical factors, nitrate and chlorophyll a concentrations in the kelp bed at Oudekraal during winter. Nitrate concentrations are from Muir (1986).

northwesterly, phytoplankton growth in waters with a high nitrate content was very rapid and chlorophyll a concentrations increased quickly (Figure 12 A,D and E). This process has been demonstrated before (Andrews and Hutchings 1980; Field et al., 1980; Brown, 1981; Carter, 1982; Brown, 1984; Brown and Field, 1986).

In winter the wind generally blew from the northwest and sea temperatures remained stable (Figure 13 A and B). It is evident from Figure 13 D and E that the pattern of fluctuations in winter chlorophyll a and nitrate concentrations in the water was similar to that occurring in summer (Figure 12 D and E), and nitrate and chlorophyll a maxima and minima were closely associated. However, in winter nitrate concentrations did not reach the same levels as in summer. Chlorophyll a levels were generally low ($\pm$ 1 $\mu g.l^{-1}$) during northwesterly gales and when the northwesterly winds relaxed, rapid increases in both nitrates and chlorophyll a occurred (Figure 13 A,D and E).

Wulff and Field (1983) modelled the kelp bed system and predicted that under continuous downwelling, phytoplankton forms 93% of the particulate matter in the kelp bed. When strong onshore northwesterly winds blow, down-welling is continuous. However, Figure 13 A and E shows that chlorophyll a concentrations in the water were low ($\pm$ 1 $\mu g.l^{-1}$) during these periods, while particulate carbon levels were fairly constant (Chapter I). It is therefore

unlikely that phytoplankton forms as much as 93% of the particulate load during these periods, and macroalgal particulates resuspended detritus probably form the major carbon source.

It is well known that increases in nitrates are the result of upwelling in summer, but the processes resulting in increases in winter nitrate levels are not as well understood. Generally winter periods of increased nitrate concentration and consequent chlorophyll a increases were closely associated with a change in wind to a southerly direction (Figure 13 A,D and E). However the change in wind direction often only lasted for a day and wind speeds were very much lower than those of the summer southeasterly winds (Figure 13 A). Also, except for days 7-9, very little change in sea temperature resulted from the brief periods of southerly wind (Figure 13 B). Thus it is unlikely that increased nitrate concentrations in winter are the result of upwelling. Furthermore, winter storms prevent the the build-up of strong gradients in nutrients (Brown, 1984) and the South Atlantic Central water mass, from which water upwells in summer, moves down the continental shelf in winter (Bang, 1973), thus reducing the likelihood of upwelling. Winter nitrate and chlorophyll a maxima were often associated with periods of large swell size (Figure 13 C,D and E). These waves resuspend bottom sediments and this may release nutrients to the water column, resulting in rapid phytoplankton growth. However, a stepwise multiple

correlation analysis of the effects of wind, temperature and swell on chlorophyll a concentrations showed only that in summer 17% of the variability in chlorophyll a concentrations was explained by swell size, and in winter none of these variables was significantly correlated with chlorophyll a concentrations at the 95% confidence level. Thus the phytoplankton food supply is not affected by variations in the physical environment in the same way as the particulate carbon resource. Particulate carbon in the water column is significantly correlated with wind and temperature in summer and wind and swell in winter (Chapter I). Further insight into the nature of the phytoplankton particulate component can be gained from examining other pigments in the particulate material.

Other pigments in particulate material

a) Phaeopigments and Phaeophytin

The Lorenzen (1967) method of chlorophyll analysis allows an estimate to be made of "phaeopigments" in the water column. These phaeopigments consist of phaeophorbides and phaeophytins and can be quantified by HPLC. Figures 14 B and 15 B show daily summer and winter phaeopigment estimates by the Lorenzen (1967) method, and phaeophytin a estimates made by HPLC from the same samples. Phaeophorbide a occurred in 15% of summer and 21% of winter samples (Table 9) and peak areas were very small and are thus not included in HPLC phaeopigments. Mean summer and winter concentrations

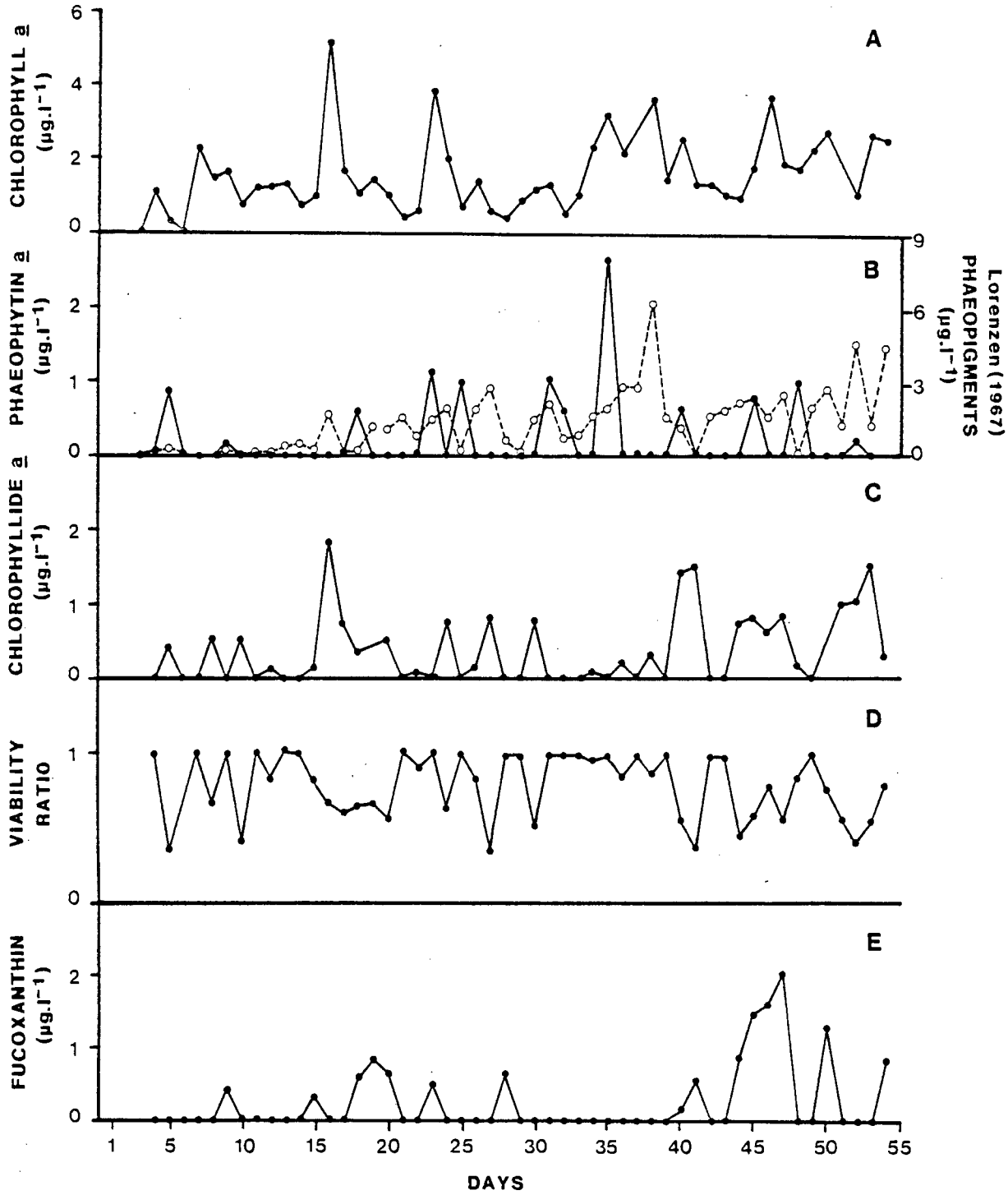


Figure 14. HPLC estimates of different algal pigment concentrations in the kelp bed water column during summer. Phaeopigment concentrations determined by the method of Lorenzen (1967) are shown (o----o) as well as the viability ratio $\frac{[\text{Chl } a]}{[\text{Chl } a] + [\text{Chlorophyllide } a]}$ where [ ] represents the molar concentration.

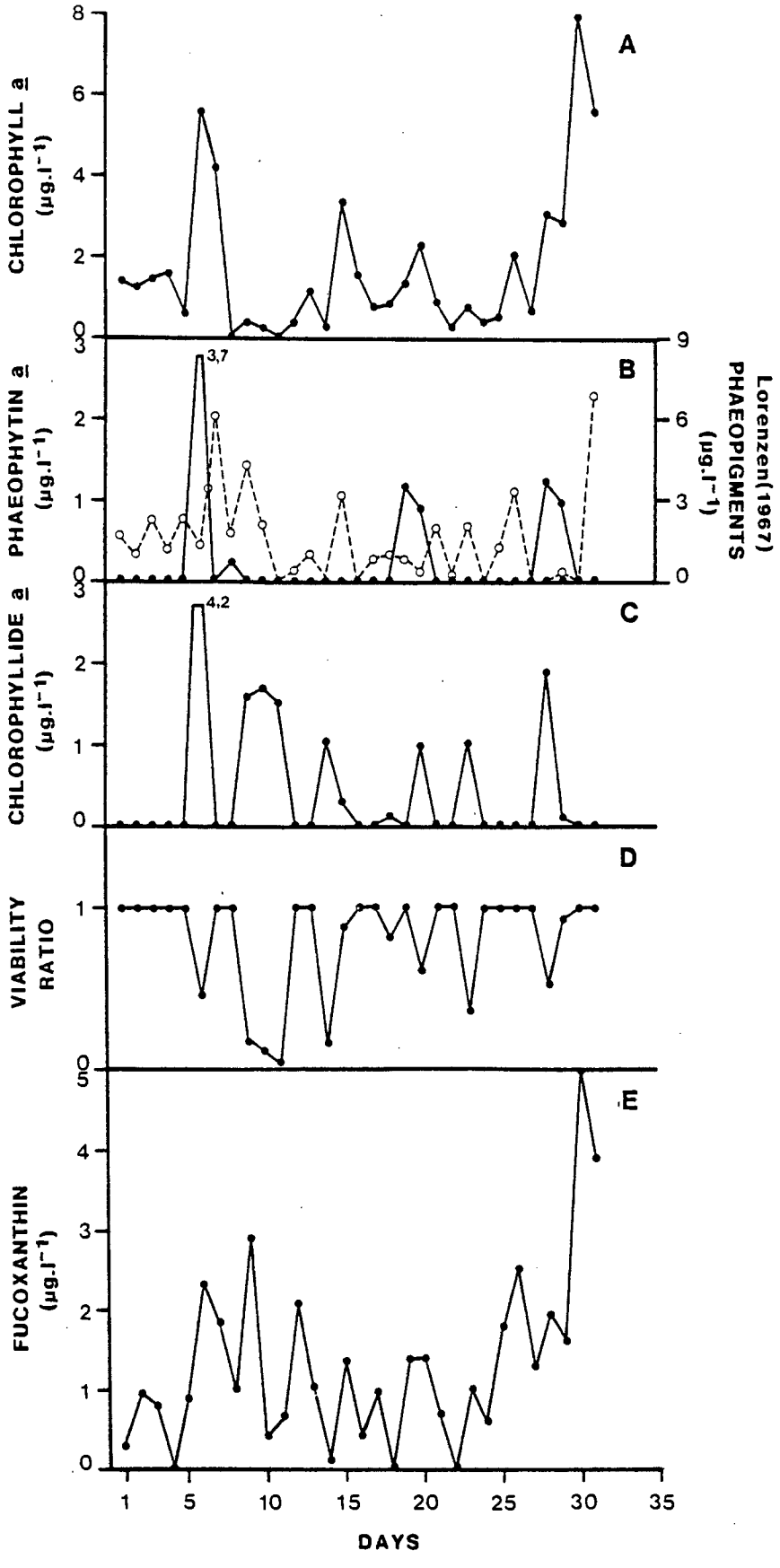


Figure 15. HPLC estimates of different algal pigment concentrations in the kelp bed water column during winter. Phaeopigment concentrations determined by the method of Lorenzen (1967) are shown (o----o) as well as the viability ratio $\frac{[\text{Chl a}]}{[\text{Chl a}] + [\text{Chlorophyllide a}]}$ where [ ] represents the molar concentration.

TABLE 9

Percentage occurrence of various minor pigments in particulate material filtered from the kelp bed water column in summer and winter.

Pigment	Summer	Winter
Chlorophyll *b*	40%	29%
Chlorophyll *c*	33%	93%
Lutein	20%	39%
Phaeophorbide *a*	15%	21%

determined by the two methods are shown in Table 10. There was a significant difference between phaeopigment concentrations determined by the Lorenzen (1967) method and phaeophytin a concentrations determined by HPLC (paired t-test, $p < 0,001$). The Lorenzen (1967) method indicated persistent phaeopigments in the water column, both in summer and winter, reaching concentrations of 7,04 $\mu g.l^{-1}$. Analysis by HPLC indicated that there were considerable periods when no phaeophytins were present and on only two occasions did concentrations exceed 2 $\mu g.l^{-1}$ (Figures 14 B and 15 B). The difference in concentrations of degradation products estimated by the two methods may in part be accounted for by carotenoid interference (mainly fucoxanthin) which may result in an overestimation of the Lorenzen (1967) values (Riemann 1982). Fucoxanthin occurred in many of the samples, particularly in winter (Figures 14 E and 15 E).

Increases in phaeopigment concentrations determined by the Lorenzen (1967) method generally coincided with increases in chlorophyll a concentrations, (Figures 14 A and B, 15 A and B) but there was no significant correlation between chlorophyll a and phaeopigment concentrations ($p > 0,05$, $r = 0,26$, $n = 52$ in summer and $r = 0,09$, $n = 31$ in winter). The appearance of phaeophytin a determined by HPLC was also often accompanied or immediately preceeded by high or rapidly increasing chlorophyll a concentrations (Figure 14 A and B, 15 A and B). HPLC chlorophyll a and phaeophytin concentrations were significantly correlated in winter

TABLE 10

Auxiliary pigment concentrations (± SD) in the kelp bed water column during summer and winter

Pigment	Summer concentration $\mu g.l^{-1}$	Winter concentration $\mu g.l^{-1}$
Phaeophytin *a*	0,21 (± 0,49)	0,27 (± 0,74)
Lorenzen phaeopigments	1,44 (± 1,37)	1,67 (± 1,75)
Chlorophyllide *a*	0,39 (± 0,49)	0,47 (± 0,93)
Fucoxanthin	0,25 (± 0,49)	1,34 (± 1,16)

($p < 0,05$, $r = 0,39$, $n = 31$) but not in summer ($p > 0,05$, $r = 0,16$, $n = 52$). Chlorophyll *a* is converted to phaeophytin *a* by the removal of the central magnesium ion through acidification and this is often the result of bacterial enzymatic digestion or zooplankton grazing (Gieskes *et al.*, 1978; Hallegraeff, 1981; Lehman, 1981; Bacon, 1984). The low phaeophytin *a* concentrations that prevail for extended periods in summer and winter (Figures 14 B and 15 B) indicate that most of the phytoplankton may be available for filter feeder consumption.

b) Chlorophyllide *a*

Figures 14 C and 15 C show the presence of this pigment during the time series. Mean summer and winter concentrations are shown in Table 10. Mean summer and winter concentrations of chlorophyllide *a* were higher than those of phaeophytin *a* (Table 10) and chlorophyllide *a* occurred in 57% of summer samples and 43% of winter samples, while phaeophytin *a* occurred in 24% of summer and 21% of winter samples. Thus it appears that chlorophyllide *a* was a more prevalent chlorophyll breakdown product than phaeophytin *a*, and chlorophyllide *a* was often present in the water column when phaeophytin *a* was absent (Figures 14 B and C, 15 B and C). Since chlorophyll *a* and chlorophyllide *a* have identical absorption spectra and are thus indistinguishable spectrophotometrically, this has important implications when algal biomass is assessed by this method. Generally the appearance of chlorophyllide *a* in the water column was not

closely related to chlorophyll a concentrations, although chlorophyllide a might be expected to appear in senescent phytoplankton populations (Jensen and Sakshaug, 1973; Jeffrey, 1974; Hallegraeff, 1981; Monteiro et al., 1986). In summer, increases in chlorophyllide a concentrations often occurred with or shortly after peaks in chlorophyll a concentrations (Figure 14 A and C), and chlorophyllide a and chlorophyll a were significantly correlated ($p < 0,01$, $r = 0,37$, $n = 52$). Winter peaks in chlorophyll a concentrations were sometimes associated with increases in chlorophyllide a concentrations, but there was no significant correlation ($p > 0,05$ $r = 0,19$ $n = 31$; Figure 15 A and C).

Monteiro et al., (1986) recommend the calculation of a viability ratio for algal populations, whereby the ratio of chlorophyll a to total chlorophyll like pigments is estimated, in order to assess the physiological status of the phytoplankton assemblage. A viability ratio

$$\frac{[\text{chlorophyll a}]}{[\text{chlorophyll a}] + [\text{chlorophyllide a}]}$$

was calculated where [chlorophyll a] and [chlorophyllide a] represent the molar pigment concentrations during the sampling period. This is shown in Figures 14 D and 15 D. Ratios close to 1,0 indicate algal populations in an actively growing phase, while low ratios indicate senescence Generally, summer and winter viability ratios were high, indicating actively growing phytoplankton. Lowered viability ratios occurred approximately one to three days after peak

chlorophyll a concentrations, showing senescence of the bloom caused by decreased nitrate concentrations. Summer nitrate concentrations reached 20-30 µg at NO_3-N.l^{-1} during upwelling but declined very rapidly within two or three days (Figure 12 D), and nitrate rather than phosphate or silicate was the limiting nutrient in the kelp bed during this time (Probyn, unpublished data).

c) Fucoxanthin

Daily fucoxanthin concentrations in the water column during summer and winter are shown in Figures 14 E and 15 E and mean summer and winter concentrations are shown in Table 10. Fucoxanthin occurred in 91% of winter samples and 30% of summer samples. Winter concentrations reached 5,35 µg.l^{-1} while the highest concentration recorded in summer was 2,04 µg.l^{-1}. Fucoxanthin is a pigment characteristic of the Bacillariophyta and Phaeophyta (Morris 1971; Parsons et al., 1984). Thus in winter, much of the particulate component in the water column was probably of diatom and kelp origin. However, since winter C:N ratios are 9,90 and approach those for kelp in winter (11,70, Dieckmann, 1978; see Chapter I), much of this material was probably of kelp origin. In summer, fucoxanthin was less frequently represented in the particulate component. Particulate matter originating from the macrophytes was probably less in summer because the reduced wave action (Figure 12 C) resulted in less frond erosion and fragmentation. However the differences in both the occurrence and concentrations of fucoxanthin in summer

and winter suggests that summer phytoplankton populations were frequently not dominated by diatoms. Figure 15 A and E also shows that in winter fucoxanthin maxima occurred at the same time as chlorophyll *a* maxima, and fucoxanthin and chlorophyll *a* were significantly correlated ($p < 0,001$, $r = 0,76$, $n = 31$). In summer the two pigments were not significantly correlated ($p > 0,05$, $r = 0,19$, $n = 52$), and except for the last nine days of the sampling period, fucoxanthin maxima occurred at different times to chlorophyll maxima (Figure 14 A and E). This implies that in summer, even during phytoplankton blooms resulting from upwelling, diatoms were not dominant in the kelp bed phytoplankton. Diatoms have generally been considered the dominant phytoplankton forms in the west coast kelp bed and inshore areas (Andrews and Hutchings, 1980; Field *et al.*, 1980; Barlow, 1982; Olivieri 1983; Probyn, 1985; Brown and Field, 1986), although Probyn (1985) recorded very high numbers of nanoflagellates in the west coast inshore area. It is possible that often these organisms are not preserved intact for counting purposes (Brown and Field, 1986). Chlorophyll *b*, characteristic of the Chlorophyta, Prasinophyta and Euglenophyta (Raymont, 1980; Parsons *et al.*, 1984), occurred more commonly in summer samples (40%) than in winter samples (29% Table 9). Flagellate forms from these three algal groups may have comprised a considerable proportion of summer phytoplankton populations, since the reduced wave action in summer (Figure 12 C) would not have favoured fragmentation of the understorey green macrophytes.

d) Minor pigments

The presence of minor pigments in the water column in summer and winter is shown in Table 9. Chlorophyll *c*, a characteristic pigment of the Bacillariophyta and Phaeophyta, occurred in 93% of winter samples and 33% of summer samples. Together with the data on fucoxanthin concentrations, the presence of chlorophyll *c* provides strong evidence that the winter particulate resource was comprised mainly of diatoms and brown algal particles, and was qualitatively different from the summer particulate resource. Lutein occurs in the Chlorophyta, Phaeophyta and Rhodophyta but was not a major pigment in the particulate fraction. Phaeophorbide *a* was present in 15-21% of samples. Since this chlorophyll breakdown product is probably a primary indicator of feeding and digestive processes of bivalves and copepods (Jeffrey, 1974; Hallegraeff, 1981; Lehman, 1981; Mantoura and Llewellyn, 1983; Hawkins *et al.*, 1986), and mussels comprise a large component of the fauna in the kelp bed system (Newell *et al.*, 1982), much of the diet of these filter feeders may consist of particulates which do not contain chlorophyll *a* and are therefore of a detrital nature.

Carbon : Chlorophyll a ratios and Bacterial relationships

Organic carbon : chlorophyll *a* ratios have sometimes been used to determine the standing stock of phytoplankton (Lorenzen, 1968; Andrews and Hutchings, 1980). Andrews and

Hutchings (1980) propose carbon : chlorophyll a ratios of 70 in summer and 140 in winter for the Cape west coast. Banse (1977) strongly cautions against the use of such ratios, on the grounds that there are often appreciable amounts of non-algal organic carbon in the particulates filtered from the water. Organic carbon : chlorophyll a (HPLC) ratios in the kelp bed fluctuated greatly, reaching values of 4422. Mean values were 344 (± 533) in summer, and 742 (± 1020) in winter. These values are very much higher than those proposed by Andrews and Hutchings (1980), and because of their great variablity, they are not a reliable indication of the amount of particulate food present in the system. The correlation between organic carbon and nitrogen, and chlorophyll a can however provide a useful indication of the type of particulate matter in the water. A high correlation coefficient indicates that much of the carbon and nitrogen present derives from actively growing phytoplankton, whereas a low correlation coefficient indicates a high proportion of detrital carbon and nitrogen. The relationship between chlorophyll a and organic carbon and nitrogen in the water is shown in Figures 16 A and B, 17 A and B. It is evident that much of the carbon and nitrogen available throughout the year was of a detrital nature, since the correlations were poor.

The relationship between chlorophyll and bacteria is not often examined when assessing particulate food sources for filter feeders. Bacteria are a possible food source for mussels living in the kelp bed (Seiderer et al., 1984; Stuart

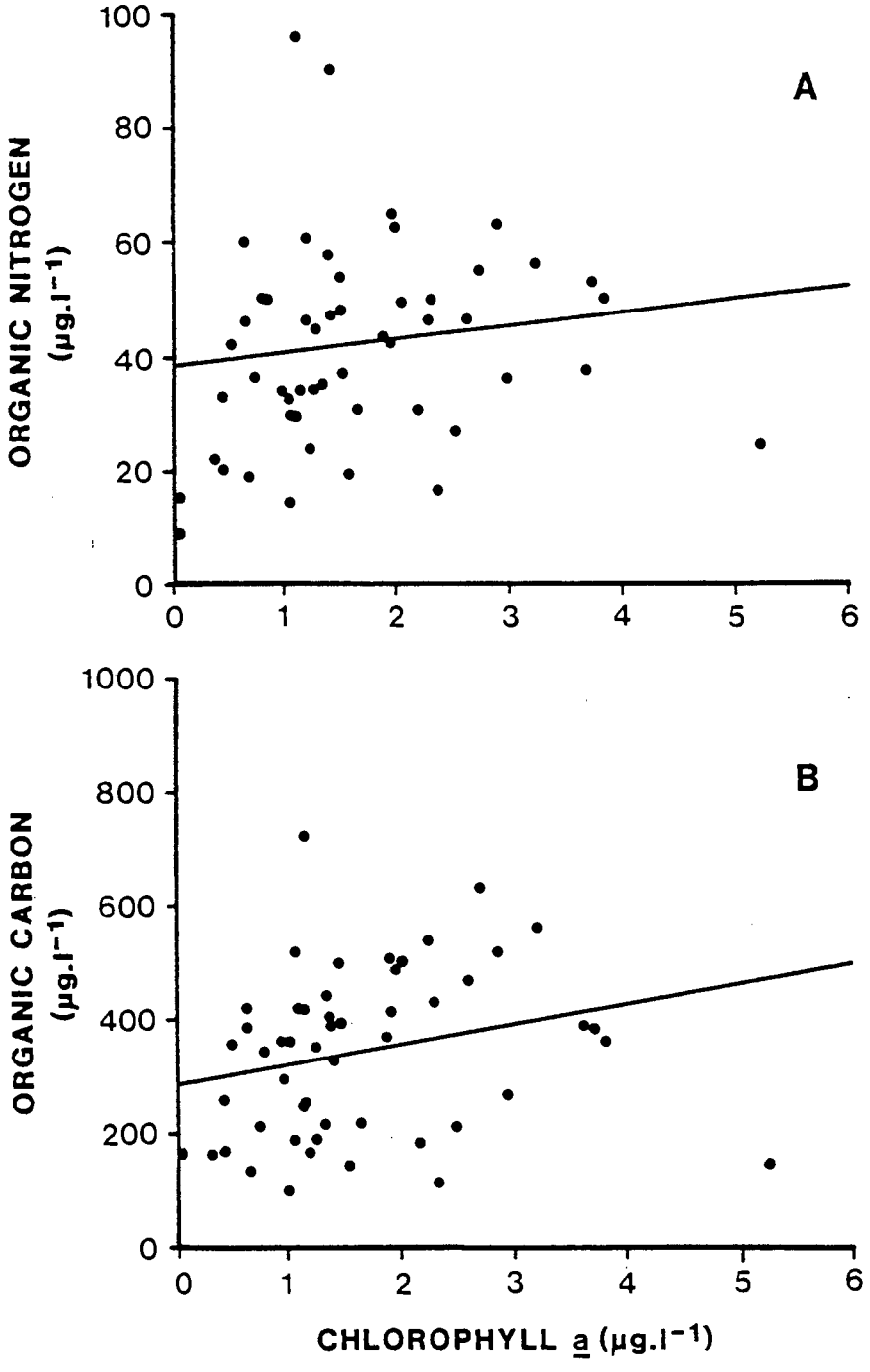


Figure 16. The relationship between summer organic carbon and nitrogen concentrations and chlorophyll a concentrations in the kelp bed. Correlation coefficient for carbon = 0,27 (n=52) and for nitrogen = 0,19 (n=52).

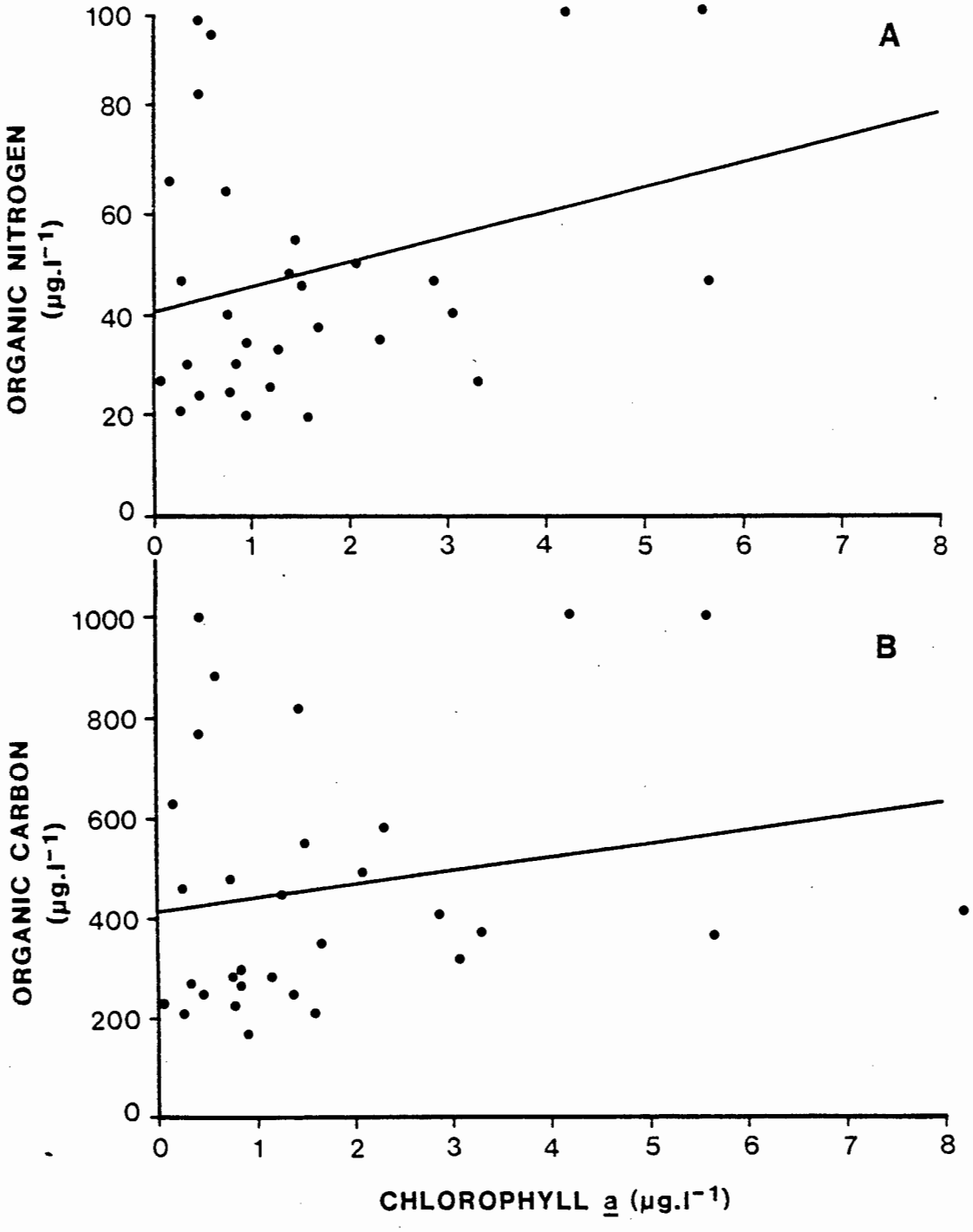


Figure 17. The relationship between winter organic carbon and nitrogen concentrations and chlorophyll a concentrations in the kelp bed. Correlation coefficient for carbon = 0,08 (n=31) and for nitrogen = 0,16 (n=30).

and Klumpp, 1984; Seiderer and Newell, 1985; Muir et al., 1986), although the bacterial contribution in terms of the total carbon resource is probably very small. The mean organic content of the water column was 380 $\mu gC.l^{-1}$ and 43 $\mu gN.l^{-1}$ (Chapter I). The mean bacterial biomass during the whole sampling period was 34 $\mu g.l^{-1}$ (Muir, 1986). Assuming a carbon equivalent of 10% and a bacterial C:N ratio of 4 (Lucas et el., 1987), this amounts to 3,40 $\mu gC.l^{-1}$ and 0,85 $\mu gN.l^{-1}$, which is less than 1% of the particulate carbon resource and approximately 2% of the particulate nitrogen resource. Since bacteria are filtered by mussels with an efficiency of approximately 20% (Stuart and Klumpp, 1984; Lucas et al., 1987), freeliving bacteria represent a very small fraction of the food resource available to the mussel population. However bacteria will also colonize detrital particles thus improving their nitrogen content (Newell et al., 1980a; Newell, 1981; Newell and Lucas, 1981; Newell et al., 1981; Stuart et al., 1981) and bacterial aggregates will be more easily retained by mussels. Figures 18 and 19 show bacterial biomass and HPLC chlorophyll a concentrations in the water column in summer and winter. Bacterial biomass is from Muir (1986). Increases in the biomass of bacteria closely followed the development of phytoplankton blooms. Phytoplankton release between 4% and 30% of fixed carbon as dissolved exudates (Jensen,1983) which are utilized by the microheterotrophic community, and thus may be the principal determinant of bacterial activity in the system (Muir, 1986). Factors affecting the phytoplankton stocks therefore have a

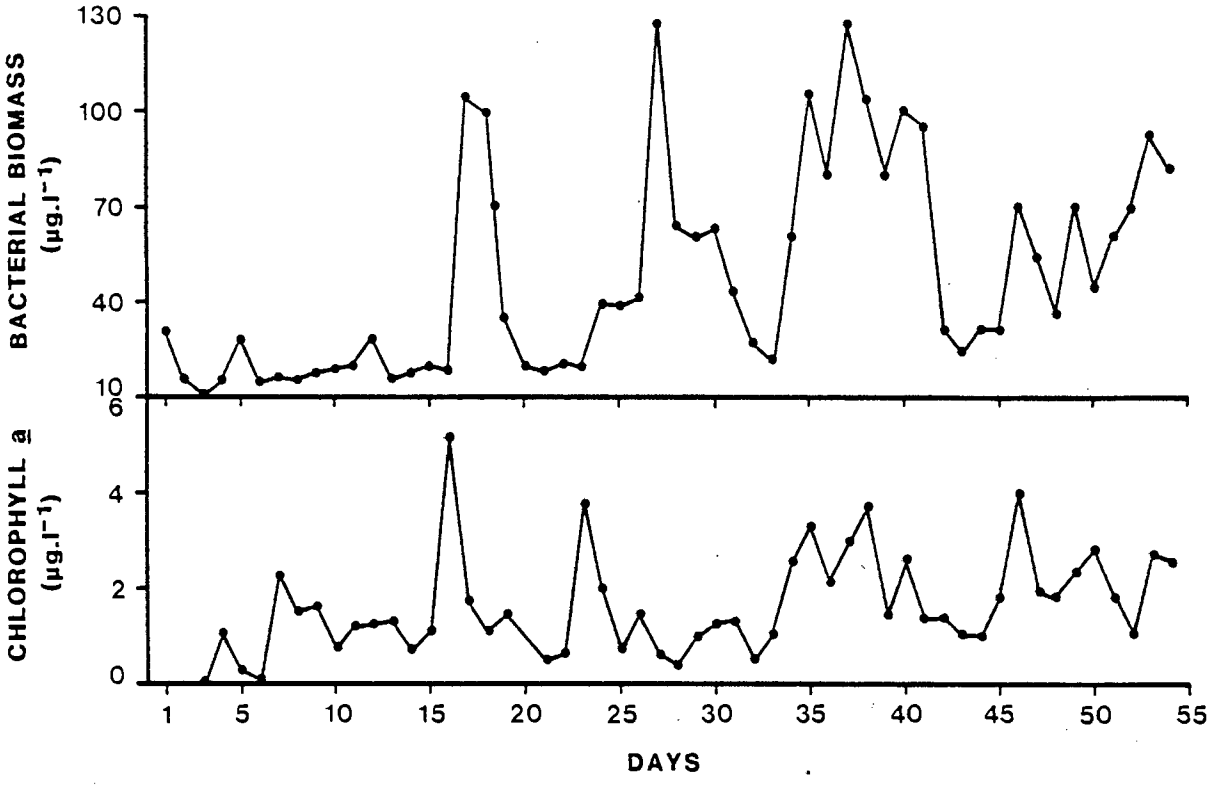


Figure 18. Summer bacterial biomass (wet) and chlorophyll a concentrations in the kelp bed. Bacterial biomass is from Muir (1986).

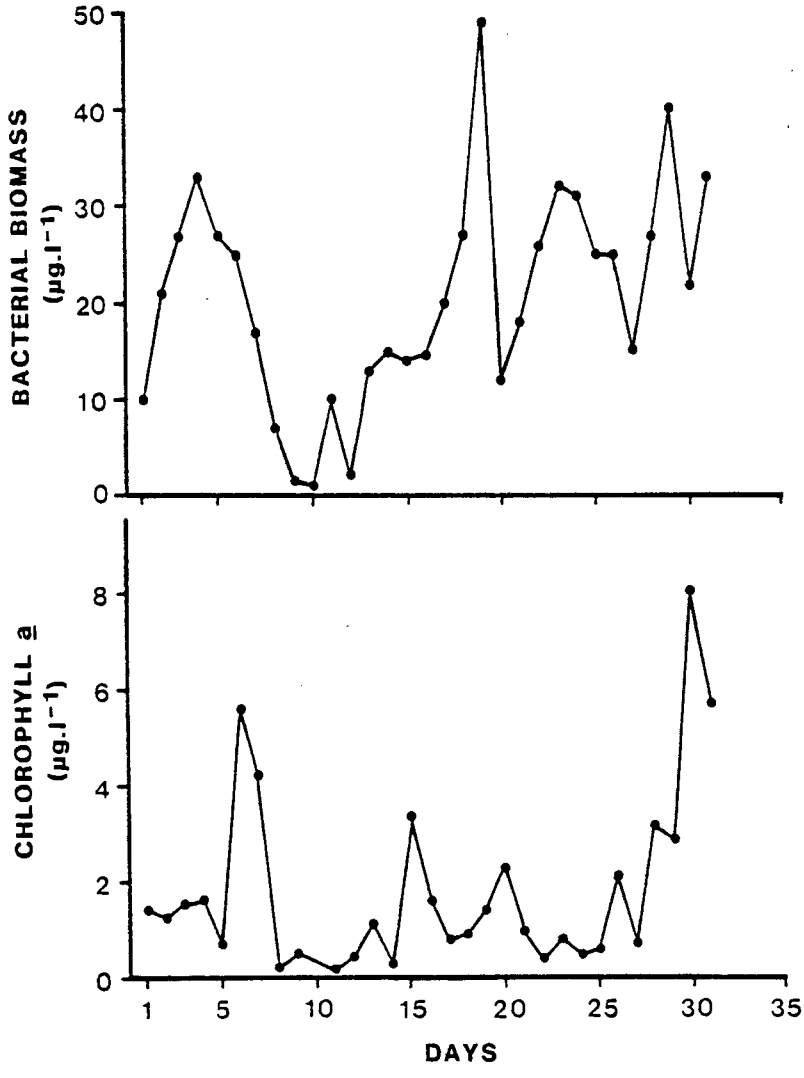


Figure 19. Winter bacterial biomass (wet) and chlorophyll a concentrations in the kelp bed. Bacterial biomass is from Muir (1986).

direct bearing on the growth of bacterial populations, whose chief importance is probably the colonization of a largely detrital particulate resource.

CONCLUSIONS

These results have shown that there was a significant difference in chlorophyll a concentrations determined by HPLC and spectrophotometric determinations, and previous work in the kelp bed area has often overestimated chlorophyll a concentrations. However, these differences were not excessive because the dynamic nature of the kelp bed system ensures regular nutrient replacement for an actively growing phytoplankton population, and allows little time for chlorophyll degradation products to accumulate. Changes in chlorophyll a concentrations were closely linked to changes in nitrate concentrations resulting from wind driven upwelling of cold, nutrient rich waters in summer, but phytoplankton populations are not correlated with environmental variables in the same way as particulate organic carbon concentrations. Seasonally, the mean values and patterns of fluctuations of chlorophyll a concentrations were very similar.

Low phaeophytin a concentrations indicated that much of the phytoplankton in the kelp bed system is available as food for

filter feders, but the lack of phaeophorbide in most samples suggests that these animals are feeding mainly on detrital particles. High concentrations of fucoxanthin, and the presence of chlorophyll c, indicated that winter particulate loads were dominated by diatoms and kelp fragments while in summer a greater proportion of green algal groups were present. There may therefore be seasonal differences in the food value of the phytoplankton fraction because of the differing chemical composition and storage products of various algal groups, and the ease with which naked flagellates, for example, may be digested, compared with diatoms. However, high carbon : chlorophyll a ratios and poor correlations between organic carbon and nitrogen, and chlorophyll a, in both summer and winter, indicated that most of the particulate fraction available to filter feeders was comprised of detrital material. Bacteria comprise a very small proportion of the particulate organic carbon and nitrogen resources in the kelp bed, but may be importatant in improving the nitrogen content of detrital material. Changes in bacterial numbers in the water column closely followed changes in chlorophyll a concentrations.

SECTION II

TESTS ON MUSSEL STYLE AND DIGESTIVE GLAND ENZYME ACTIVITIES

Energy balance studies on kelp bed mussels based on their clearance rates, absorption efficiencies, respiration losses and the energy available as primary production have consistently shown that metabolic costs of the mussels can be comfortably met by the ingested ration (Chapter 1, and see also Stuart, 1982; Bayne et al., 1984; Stuart and Klumpp, 1984; Seiderer and Newell, 1985). A few studies have also attempted to relate bivalve energy expenditure with energy gained from the hydrolysis of ingested material by digestive enzymes (Seiderer et al., 1982; Lucas and Newell, 1984; Harris, unpublished data). It is conspicuous however, that estimates of energy released from natural detrital material by style enzymes can account for no more than 30-40% of bivalve energy requirements (Lucas and Newell, 1984; Harris, unpublished data). An obvious failing is that digestive gland enzymes have not been considered. Furthermore, it has become clear that the methods used to assay the products of mussel enzyme hydrolysis are open to criticism on a quantitative basis (Farouki and Gunn, 1983; Rivers et al., 1984; Breuil and Saddler, 1985; Harris, unpublished data).

In this section an attempt is made to critically evaluate the methods for assaying the products of enzyme hydrolysis, and the ability of mussel style and digestive gland enzymes to hydrolyse purified commercial substrates is tested. This approach removes the ambiguity of having substrate quality as an unknown variable, as would be the case if natural food resources were used in these tests.

CHAPTER III

IMPLICATIONS FOR THE ASSESSMENT OF CRYSTALLINE STYLE ACTIVITY IN BIVALVES WHEN USING THE BERNFELD AND NELSON-SOMOGYI ASSAYS FOR REDUCING SUGARS.

INTRODUCTION

It has been reported that the crystalline style of bivalves contains digestive enzymes and therefore plays a role in extracellular digestion (Yonge, 1923). Since Coupin (1900) demonstrated the presence of α-amylase in the style of Cardium sp., numerous studies have supported this evidence (Lavine, 1946; Kristensen, 1972a; Seiderer and Newell, 1979; Newell et al., 1980b). The presence of other carbohydrases such as laminarinase (Bull and Chesters, 1966; Sova et al., 1970; Shallenberger et al., 1974), alginate lyase (Jacober et al., 1980; Muramatsu and Egawa, 1980) and cellulase (Stone and Morton, 1958; Yokoe and Yasumasu, 1964; Horiuchi and Lane, 1965, 1966; Koopmans, 1970; Elyakova, 1972) have also been reported. Lysozymes have been isolated from the styles of both Mytilus edulis (McHenery and Birkbeck, 1979, 1982) and Choromytilus meridionalis (Seiderer et al., 1984).

The presence of carbohydrases suggests that bivalves are capable of utilizing structural carbohydrates, including those from detritus (Seiderer et al., 1982). Attempts have been made to relate available food to the presence of carbohydrases, and conclusions indicate that cellulolytic activity corresponds to the level of cellulose in the diet (Crosby and Reid, 1971). In a study of the carbohydrases of some marine invertebrates, Kristensen (1972a) found that the highest incidence of carbohydrases occurs in detrital feeders and microherbivores. More recently quantitative estimates of

the energy released from crystalline style activity have been compared with the energy requirements of a number of bivalves (Seiderer et al., 1982; Lucas and Newell, 1984).

Sugars resulting from enzymatic hydrolysis of carbohydrates by crystalline style preparations have been detected by various methods. These include turbidometric determinations (Crosby and Reid, 1971; Muramatsu et al., 1977), radiochemical assays (Smucker and Wright, 1984), chromatographic methods (Kristensen, 1972a; Pernas et al., 1981) and glucose test kits (Horiuchi and Lane, 1965; Jacober et al., 1980). Viscosimetric methods of estimating enzyme activity have often been used (Yokoe and Yasumasu, 1964; Horiuchi and Lane, 1965,1966; Okado et al., 1966; Muramatsu et al., 1977), but the most widely used methods are spectrophotometric (Galli and Giese, 1959; Horiuchi and Lane, 1965,1966; Okado et al., 1966; Sova et al., 1970; Kristensen, 1972a; Wojtowicz,1972; Alemany and Rosell-Perez, 1973; Lindley et al.,1976; Rosiou and Iacovache, 1980; Seiderer and Newell, 1979; Newell et al., 1980b; Trainer and Tillinghast, 1982; Lucas and Newell, 1984).

Glucose oxidase-peroxidase (Horiuchi and Lane, 1966; Crosby and Reid, 1971) and KI-KIO3 (Alemany and Rosell-Perez, 1973) reagents have been used to develop a colour in response to low molecular weight carbohydrates resulting from the action of carbohydrases on complex carbohydrates. Two of the methods most widely used in molluscan style enzyme experiments are

the Nelson-Somogyi test for reducing sugars (Nelson, 1944; Somogyi, 1952) and the Bernfeld test for reducing sugars (Bernfeld, 1955; from Sumner, 1921, 1924, Sumner and Howell, 1935). The former relies on the oxidation of the free aldehyde/ketone groups of the reducing sugar, by Cu^{++} in alkaline solution, while the latter is based on the reduction of alkaline 3,5 dinitrosalicylic acid (DNS). Both Nelson-Somogyi and Bernfeld (DNS) procedures may be used to detect the presence of reducing sugars produced by the action of carbohydrases on a range of substrates eg. glycogen, starch, laminarin, cellulose. Therefore they are useful tools in examining and comparing enzymatic activities of homogenates of crystalline styles, stomachs and digestive glands.

Recently a number of studies have attempted to quantify the significance of style activity in terms of the energy requirements of the animal using the Nelson-Somogyi and Bernfeld methods. Seiderer et al., (1982) have shown that crystalline style enzymes of C. meridionalis and Perna perna can release between 4,80 and 9,63 mg glucose.mg^{-1} style protein.h^{-1} from commercial substrates and they suggest that the style enzyme may therefore probably release sufficient carbon from natural detritus and phytoplankton to meet the requirements of the animals. Lucas and Newell (1984), using naturally occurring detritus, obtained specific activities of the carbohydrases of the style of Crassostrea virginica and Geukensia demissa and found that style enzyme activity may account for 33% to 48% of the carbon requirements of the

animals. Both of these studies used the Nelson-Somogyi and Bernfeld methods for estimating reducing sugar release by crystalline style enzymes. However, in a study of the quantitative significance of the crystalline style enzymes of Mactra glabrata, in which both the Bernfeld and Nelson-Somogyi methods for estimating reducing sugars were employed, the Bernfeld method consistently indicated 4 to 6,7 times more reducing sugars present than the Nelson-Somogyi method using commercial enzymes, and approximately 2,5 times more using style enzymes. Use of these assay procedures can imply that as little as 6% of the animal's energy demand is met by style enzyme hydrolysis of naturally occurring substrates (Harris, unpublished data). Since carbon availability calculated from glucose release by style enzymes in previous studies may have been over or underestimated by a factor of about 2,5 depending on the assay procedure used, these assays need to be examined with reference to the effect they may have on inferences previously made about the ecophysiology of some bivalves. Several factors can affect the reliability of the DNS assay as an accurate analytical method for evaluating reducing sugars (Miller, 1959; Farouki and Gunn, 1983; Rivers et al., 1984; Breuil and Saddler, 1985), and quantitative differences between reducing sugars measured by the DNS and Nelson-Somogyi methods have been recently reported (Breuil and Saddler, 1985). In this chapter the discrepancy between the two methods is investigated to illustrate the significance this has on the interpretation of results for purposes of energy budget estimations. Previously calculated

energy budgets are recalculated in the light of the present data, to try and estimate the quantitative significance of style enzyme liberation of reducing sugars relative to the energetic demands of certain bivalves.

MATERIALS AND METHODS

General assay

Assays for reducing sugars released by enzymatic action on a carbohydrate substrate were conducted according to Bernfeld (1955) and Nelson (1944) modified by Somogyi (1952), as detailed in Table 11.

The results were corrected by subtracting both substrate and enzyme blanks and are expressed as mg glucose.mg enzyme^{-1} for commercial enzymes and as mg glucose.mg protein^{-1} for style enzymes. All values represent the mean of three estimates. Release of reducing sugars by enzymatic activity was tested over 21 minutes and found to be linear for at least 9 minutes, so all experiments employed a 9 minute incubation period.

Reducing sugar release was calculated from calibration equations established using glucose as a standard. The Bernfeld calibration curve established for glucose concentrations of 0,063 to 4,0 mg.ml^{-1} was $Y = 0,01 + 0,385X$

TABLE 11

Outline of the procedures of two methods for estimating release of reducing sugars by enzymes.

Method	Nelson (1944) modified by Somogyi (1952).	Bernfeld (1955)
Reagents	Somogyi reagent (A:B, 4:1)	1.3-Dinitrosalycilic acid (DNS)
Assay 16°C for 9 min. in shaking water bath	0.5ml enzyme + 0.5ml substrate	0.5 ml enzyme + 0,5 ml substrate
Procedure:		
1)To halt reaction	add 1 ml Somogyi reagent and boil for 10 mins. Cool.	add 0.5 ml DNS and boil for 5 mins. Cool.
2)Colour development	add 2ml Nelson reagent and 6 ml H_2O	add 10 ml H_2O
3)Absorbance	read at 660nm	read at 540nm

(r^2 = 0,98, n = 7) where Y is the absorbance at 540 nm and X is the concentration of β-D(+) glucose in $mg.ml^{-1}$. The Nelson-Somogyi calibration curve for glucose concentrations of 0,0015 to 0,188 $mg.ml^{-1}$ was Y = -0,03 + 14,73X (r^2 = 0,99, n = 7), where Y is the absorbance at 660 nm and X is the concentration of B-(D+) Glucose in $mg.ml^{-1}$.

Substrate Preparation

Substrates were prepared in 20mM phosphate buffer (pH 6,9) containing 150 mM NaCl, at the following concentrations: Oyster glycogen (BDH No. 38042) 1% w/v, starch (BDH No. 30264) 1% w/v, carboxymethyl cellulose (CMC, BDH) 1% w/v, laminarin (Sigma No. L9634) 0,4% w/v.

Enzyme Preparation

Bacterial α-amylase from Bacillus subtilus (Sigma, bacterial crude type 3) was prepared in phosphate buffer pH 6,9 at a concentration of 0,25 $mg.ml^{-1}$ and composite cellulase from Aspergillus niger (Sigma practical grade 2) was dissolved in phosphate buffer (pH 6,9) at a concentration of 0,5 $mg.ml^{-1}$. Solutions of mussel crystalline style enzymes were prepared from the styles of C. meridionalis as described by Seiderer et al., (1982). Protein concentrations of the style enzyme solutions were determined according to the method of Lowry et al., (1951).

Test for Enzyme/Substrate Interference in Assay

A series of tests were conducted to determine whether either the enzyme or the substrate was interfering with reactions taking place during Bernfeld and Nelson-Somogyi reducing sugar assays. Glucose (0,199 $mg.ml^{-1}$), α-amylase (0,25 $mg.ml^{-1}$) and oyster glycogen (1% w/v) were made up in phosphate buffer (pH 6,9). As the reaction equation was considered to be Enzyme + Substrate ---> Reaction Products, an equivalent volume of glucose solution was substituted for Enzyme + Substrate, and then for each factor in turn, and analyses of reducing sugars present were conducted by both Bernfeld and Nelson-Somogyi methods.

Calibration Curves

Standard solutions of 11 saccharides (0,4 $mg.ml^{-1}$) were made up in phosphate buffer (pH 6,9). The saccharides were dextrin, starch, lactose, cellobiose, maltose, galactose, mannose, fructose, xylose, arabinose and ribose. Each stock solution (0,4 $mg.ml^{-1}$) was serially diluted so that solutions of 0,2 $mg.ml^{-1}$, 0,1 $mg.ml^{-1}$, 0,05 $mg.ml^{-1}$, 0,025 $mg.ml^{-1}$, 0,0125 $mg.ml^{-1}$, and 0,0063 $mg.ml^{-1}$ were obtained. Saccharide solutions ranging from 0,4 $mg.ml^{-1}$ to 0,05 $mg.ml^{-1}$ were assayed by the Bernfeld method as the lower limit for detection of reducing sugars by this method is approximately 0,05 $mg.ml^{-1}$. The Nelson-Somogyi method was more sensitive and saccharide solutions ranging from 0,1 $mg.ml^{-1}$ to 0,0063 $mg.ml^{-1}$ were assayed by this method. Using the Nelson-Somogyi

method, saccharide solutions with concentrations above 0,1 $mg.ml^{-1}$ approached the recommended maximum limit for accurate readings on the spectrophotometer (Beckman model 25), and 0,1 $mg.ml^{-1}$ was therefore taken as an upper limit. Phosphate buffer blanks were subtracted from experimental values and data points given in results are the mean of triplicate readings.

RESULTS

Comparison of two assay methods.

Bernfeld and Nelson-Somogyi assays of the reducing sugars produced by incubations of α-amylase, cellulase and mussel enzyme with glycogen, starch, CMC and laminarin are shown in Table 12, Reducing sugar values measured by the Bernfeld method were 4 to 6,7 times higher than those measured by the Nelson-Somogyi method for commercial enzymes, and 2,2 to 2,7 times higher for style enzymes. Tests were therefore conducted to see if the enzyme or substrate was interfering in the reaction for either method. The results of substituting a glucose solution of known concentration for either the enzyme or substrate fraction of an incubation, or both, and then assaying for reducing sugars present by the Bernfeld and Nelson-Somogyi methods are shown in Table 13. The measurements obtained for identical quantities of

TABLE 12

Comparison of the carbohydrase activities of α-amylase, cellulase and mussel style enzymes in the presence of starch, glycogen, CMC and laminarin by the Bernfeld and modified Nelson-Somogyi methods. Values represent the release of reducing sugars after 9 mins. incubation at 16°C and are expressed as mg glucose.mg enzyme^{-1}.h^{-1} for α-amylase and cellulase, and mg glucose.mg style protein^{-1}.h^{-1} for style enzymes.

Incubation	Enzyme activity		Discrepancy
	Bernfeld method	N-Somogyi method	Bernfeld / N-Somogyi
α-amylase + Glycogen	58,9	10,29	5,72
α-amylase + Starch	90,25	16,62	5,43
α-amylase + CMC	0,00	0,00	-
Cellulase + Glycogen	8,25	1,24	6,65
Cellulase + Starch	13,35	2,45	5,40
Cellulase + CMC	0,80	0,20	4,0
Style Enzyme + Glycogen	4,85	2,24	2,17
Style Enzyme + Starch	10,00	3,65	2,74
Style Enzyme + Laminarin	0,38	0,16	2,38
Style Enzyme + CMC	0,00	0,05	-

TABLE 13

Estimation of a known quantity of reducing sugar by the Bernfeld and Nelson-Somogyi methods when substrate and enzyme are present independently: (Units are mg glucose.ml^{-1}).

Incubation	Reducing sugar estimate			Discrepancy
	Bernfeld method	N-Somogyi method	Known sugar conc^{n}	Bernfeld / N-Somogyi
Glucose only	0,210	0,187	0,199	1,12
Enzyme + Glucose	0,101	0,102	0,099	0,99
Substrate + Glucose	0,116	0,098	0,099	1,18

reducing sugars were comparable for the two methods when enzyme and substrate are present independently.

Calibration Curves

Linear regressions and their equations for standard curves generated by Bernfeld and Nelson-Somogyi assays of known concentrations of 12 different saccharides, are shown in Figures 20 and 21 respectively and in Table 14. Using the Bernfeld method for the assay of various saccharides resulted in calibration curves with slopes ranging from 0,32 to 0,53 (Figure 20). Slopes of monosaccharide pentose sugars were between 0,52 and 0,53, while high molecular weight (>600) polysaccharides produced virtually no colour reaction (slope≈0). Figure 22 shows that the slopes of the Bernfeld calibration curves increased with declining molecular weight of the saccharide (r^2 = 0,89). Nelson-Somogyi determinations of the same saccharides resulted in calibration curves with slopes ranging from 5,92 to 16,34 (Figure 21). Again, a monosaccharide had the steepest slope (16,34), while high molecular weight (>600) polysaccarides produced no colour reaction.

The relationship between calibration curve slope and molecular weight of the saccharide was rather better for the Bernfeld assay (r^2 = 0,89) than for the Nelson-Somogyi assay (r^2 = 0,72; See Figure 22).

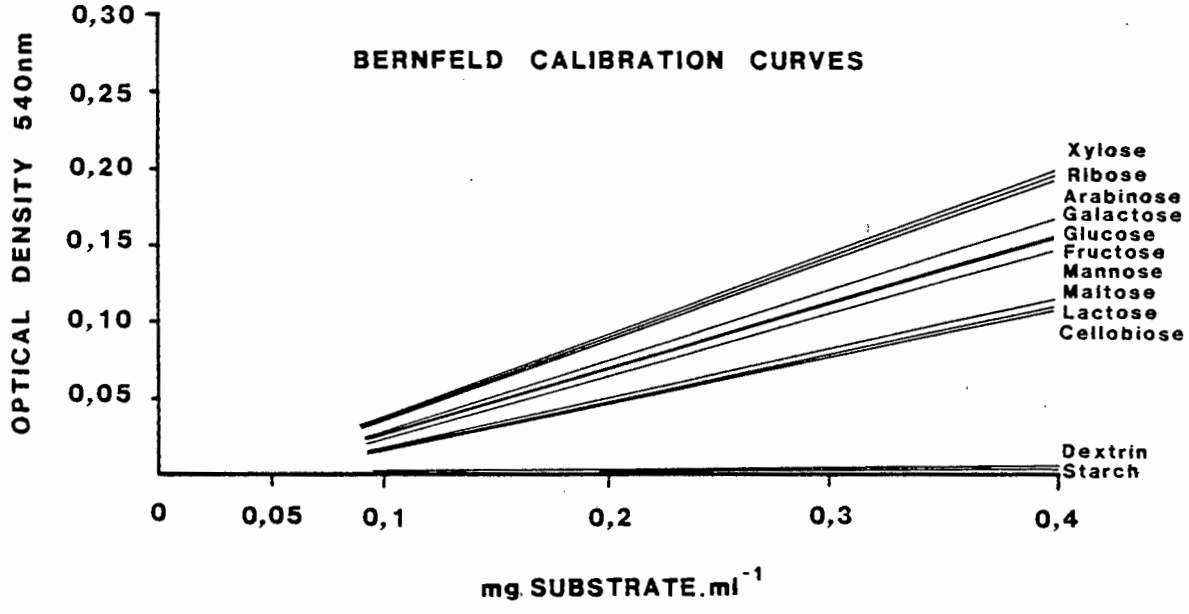


Figure 20. Calibration curves of 12 saccharides using the Bernfeld assay for reducing sugars.

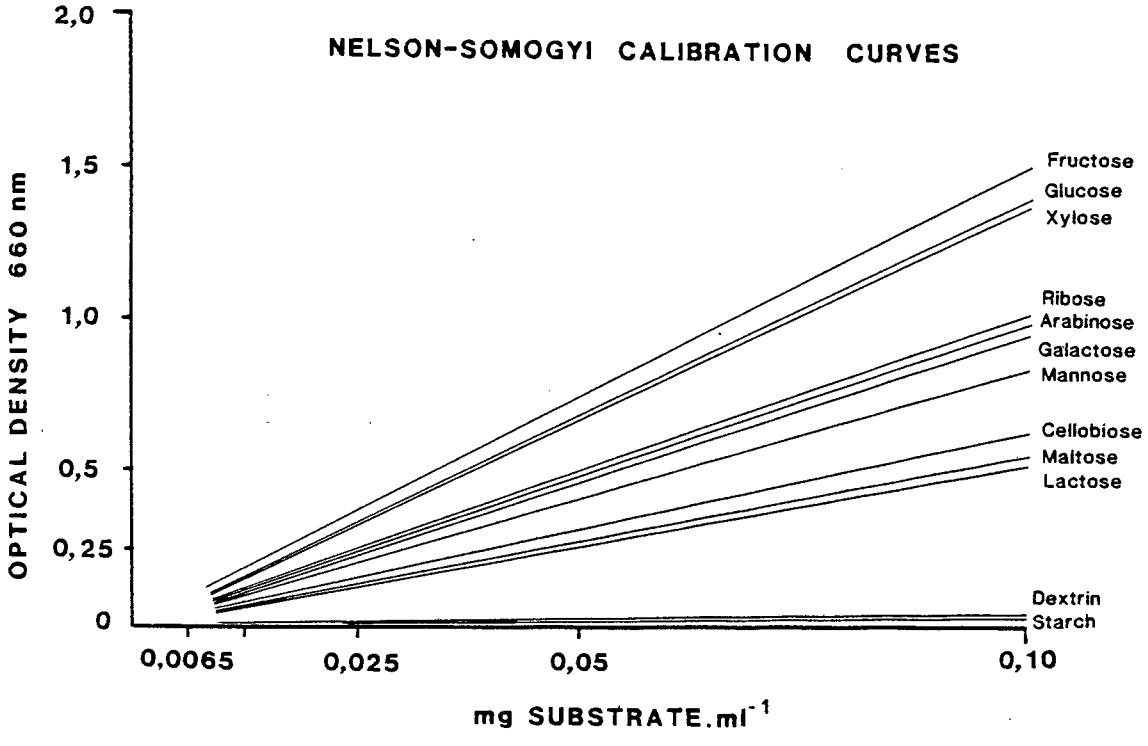


Figure 21. Calibration curves of 12 saccharides using the Nelson-Somogyi assay for reducing sugars.

TABLE 14

Standard curve regressions (Y = a + bx), and molecular weights of various saccharides using Bernfeld and Nelson-Somogyi assay procedures.

			Bernfeld Method				Nelson-Somoygi Method			
Saccharide	Type	Molec.Wt.	a	b	r	n	a	b	r	n
Ribose	Pentose	150.13	-0,02	0,53	0,99	4	-0,03	11,32	0,99	5
Xylose	Pentose	150.13	-0,01	0,53	0,99	4	-0,04	14,98	0,99	5
Arabinose	Pentose	150.13	-0,01	0,52	0,99	4	-0,06	10,75	0,99	5
Fructose	Hexose	180.16	-0,02	0,43	0,99	4	-0,02	16,34	0,99	5
Mannose	Hexose	180.16	-0,02	0,42	0,99	4	-0,02	9,16	0,99	5
Galactose	Hexose	180.16	-0,02	0,46	0,99	4	-0,03	10,70	0,99	5
Glucose	Hexose	180.16	0,01	0,39	0,98	7	-0,03	14,73	0,99	7
Lactose	Disacc.	342.30	-0,01	0,32	0,99	4	-0,02	5,92	0,99	5
Cellobiose	Disacc.	342.30	-0,02	0,32	0,99	4	-0,01	6,20	0,99	5
Maltose	Disacc.	342.30	-0,02	0,33	0,99	4	-0,02	6,24	0,99	5
Dextrin	Polysacc.	+600	-0,002	0,02	0,92	4	0,00	0,50	0,94	5
Starch	Polysacc.	+10000	0,00	0,00	0,00	4	0,002	0,004	0,10	5

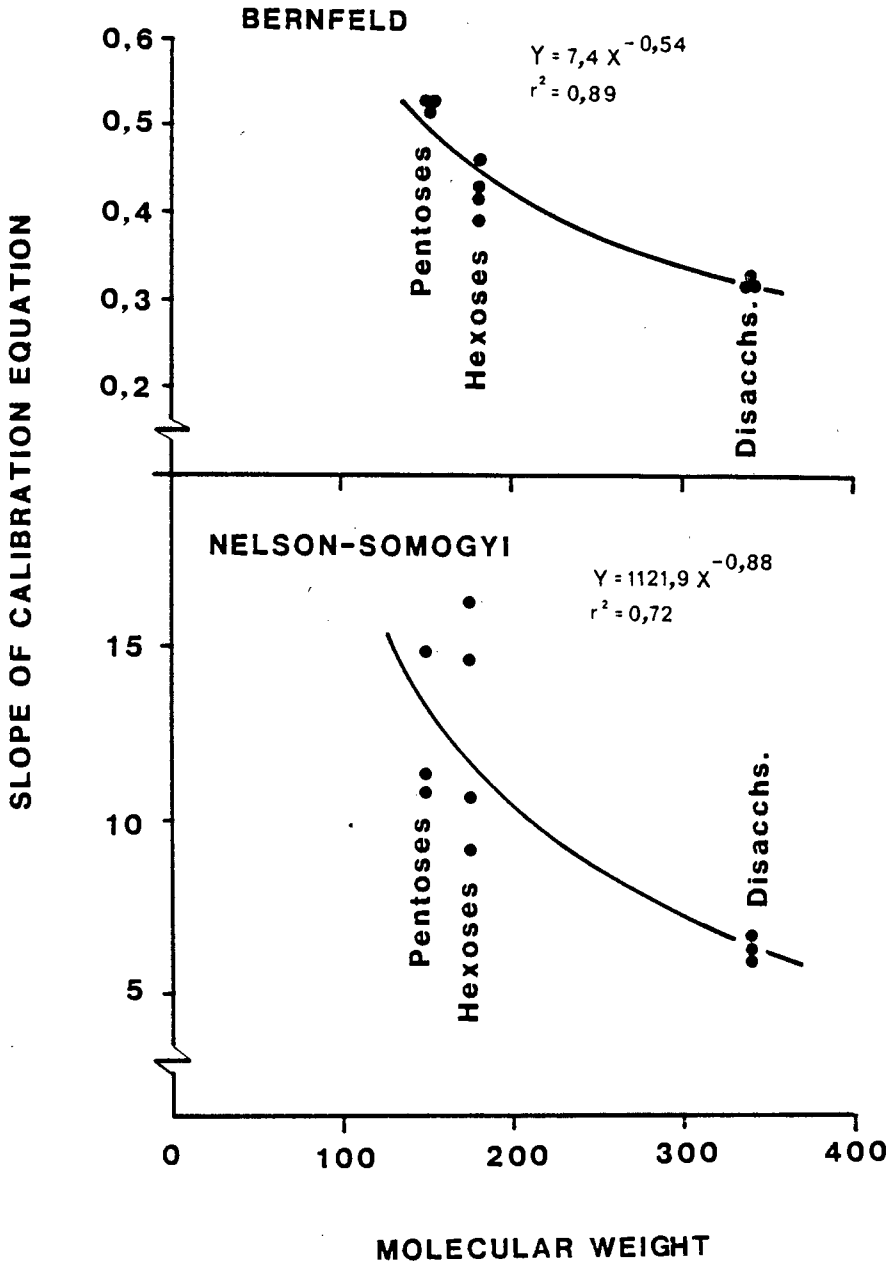


Figure 22. A plot of calibration curve slope against saccharide molecular weights for the Bernfeld and Nelson-Somogyi assays.

DISCUSSION

Response of the assay methods to different saccharides

Figures 20 and 21 show the importance of the molecular weight of saccharides in determining changes in optical density for a given concentration of reducing sugar in solution. The first derivative (dy/dx) calculated from Figure 22 for molecular weights of 150,13, 180,16 and 342,30 is at least an order of magnitude greater for the Nelson-Somogyi method than for the Bernfeld method, indicating the greater sensitivity of the former method to the molecular weights of the saccharides assayed. The varying response of the DNS method to the saccharide assayed has been reported before (Miller, 1959; Farouki and Gunn, 1983; Breuil and Saddler, 1985). The sensitivity of both methods to the molecular weight of the saccharide assayed becomes important if energy budgets are calculated from incubations of crude enzymes and substrates which produce a number of varying molecular weight end products. From Figures 20 and 21 it is evident that for both Bernfeld and Nelson-Somogyi methods, high molecular weight (>600) polysaccharides have regression slopes of virtually zero even though most polysaccharides have a terminal monomer present as a reducing sugar. Disaccharides have lower regression slope coefficients than monosaccharides, and of the monosaccharides, the pentose regression slope coefficients are greater than those of the hexoses for the Bernfeld method, although this is not so for the Nelson-

Somogyi method. The stereochemistry of the sugar molecules may significantly affect their quantitative assay by the Nelson-Somogyi method. Using this method disaccharides have similar calibration regression slopes ranging from 5,92 to 6,72, but calibration slopes for monosaccharides range from 9,16 (mannose) to 16,34 (fructose, see Table 14). However, fructose, glucose, galactose and mannose all have a molecular weight of 180,16, so it is difficult to understand why the regression slope coefficients vary so much, particularly as glucose, mannose and galactose are stereoisomers. The fact that fructose has the steepest calibration slope may be linked to the fact that it is a ketose sugar whereas all the other sugars are aldoses. Table 14 shows that using the Nelson-Somogyi method, pentose sugars also have differing calibration regression slopes (10,74 to 14,98), although all have a molecular weight of 150,13. Arabinose and ribose have similar slopes (10,74 and 11,32), but the calibration regression slope of xylose (14,98) is very similar to that of glucose (14,74). The difference in pentose calibration slope regressions may be a result of the exposed nature of the axial reducing group in xylose, whereas in arabinose and ribose the reducing group is equatorial and not quite so easily oxidised. The stereochemistry of sugar molecules does not seem to affect the Bernfeld assay procedure in any significant way (Figure 20, Table 14).

Sensitvity of assay methods.

The slopes of the mono- and disaccharide standard curves obtained for the Nelson-Somogyi method (6,24 to 14,98) were much higher than those obtained for the Bernfeld method (0,33 to 0,53; see Table 14). Thus for the former method a large change in optical density results from a small change in reducing sugar concentration, while for the latter method, a small change in optical density is the result of a large change in reducing sugar concentration. The Nelson-Somogyi method is therefore more sensitive to changes in reducing sugar concentration than the Bernfeld method. Furthermore, the Nelson-Somogyi method is also sensitive to sugar concentrations of less than 0,05 $mg.ml^{-1}$, whereas at this concentration, optical densities approach zero for the Bernfeld method (Figures 20 and 21).

Farouki and Gunn (1983), and Breuil and Saddler (1985) also demonstrate the greater sensitivity of the Nelson-Somogyi method. In addition the DNS method has been shown to be affected by the buffer used for enzyme dilution (Miller, 1959), the presence of Ca^{++} ions (Robyt and Whelan, 1972), certain mineral salts (Farouki and Gunn, 1983), enzyme concentration (Breuil and Saddler, 1985), and may possibly be affected by the degree of polymerization of the residue to which the reducing group is attached (Breuil and Saddler, 1985).

Quantitative difference between assay procedures

When reducing sugars released from the incubation of polysaccharide substrates with commercial enzymes were estimated using both the Bernfeld and Nelson-Somogyi methods, the Bernfeld method invariably gave an estimate 4 to 6,7 times higher than the Nelson-Somogyi method if glucose was used as a standard (Table 12). Furthermore, mussel crystalline style enzymes produced between 2 and 2,7 times more reducing sugars when estimated by the Bernfeld method compared with the Nelson-Somogyi method. Similarly, when investigating the action of bacterial cellulase on filter paper, Breuil and Saddler (1985) found reducing sugar estimates obtained by the dinitrosalicylic acid method were 45% higher than those obtained by the Nelson-Somogyi method. From the results presented in Table 13 it is evident also that neither the enzyme nor the substrate, when present independently, caused undue interference in the quantitative estimation of an analytical grade reducing sugar, and that both the Bernfeld and Nelson-Somogyi methods of assaying such pure sugars gave similar results. This suggests that it is the reaction products of polysaccharide hydrolysis which influence the estimate of free reducing sugars obtained using either method.

During the enzymatic hydrolysis of polysaccharides by crude enzyme extracts of molluscan digestive gland or crystalline style, intermediate compounds are produced which consist of di- and oligosaccharide units, which may be further

hydrolysed to the constituent monosaccharides that make up the major reaction products. At the time of assay by Nelson-Somogyi or Bernfeld methods, a typical hydrolysis process is in progress, and the reducing sugars measured by the two methods probably consist of a mixture of mono-, di- and oligosaccharides. Figures 20 and 21 show that with both methods, changes in optical density caused by changes in concentration of reducing sugar in solution are to a large extent dependent on the molecular weight of the sugar involved. When the enzyme-substrate reaction mixture is stopped for assay before the hydrolysis is completed, the relative proportions of the oligosaccharides, disaccharides, hexoses and pentoses will therefore affect the optical density reading. If a glucose calibration curve (Table 14) is used to determine the amount of reducing sugars present in such a reaction and only disaccharides such as maltose are present in the assay mixture, any optical density reading would provide an estimate of reducing sugars present that is 15% too low by the Bernfeld method and 58% too low if the Nelson-Somogyi method is used, since the maltose:glucose slope ratios calculated from Table 14 are 0,85 for the Bernfeld procedure and 0,42 for the Nelson-Somogyi procedure. If only pentose sugars such as arabinose are present, the estimate of reducing sugars present would be 33% too high by the Bernfeld method and 27% too low by the Nelson-Somogyi method, since the arabinose:glucose slope ratios calculated from Table 14 are 1,33 for the Bernfeld procedure and 0,73 for the Nelson-Somgyi procedure. Thus the Nelson-Somogyi

method is most likely to underestimate reducing sugars present if glucose is used for calibration purposes, and if disaccharides comprise the major part of the assay mixture, the underestimation is likely to be about 4 times greater than if the Bernfeld method is used.

Since mussel crystalline styles possess a suite of carbohydrases (Sova et al., 1970; Kristensen, 1972a; Gianfreda et al., 1979; Seiderer et al., 1982) a more complete hydrolysis of complex polysaccharides to component dimers or monomers is to be anticipated. This is likely to give better agreement between the two methods, as is observed when an analytically pure sugar is assayed (Table 13). This would account for the reduced discrepancy between the two methods when commercial substrate hydrolysis by style enzymes rather than commercial enzymes is assayed (Table 12), particularly if the latter are capable only of partial substrate hydrolysis.

If an incubation is allowed to continue until all poly-, oligo- and disaccharides are reduced to monosaccharides, then the two methods should provide similar quantitative estimates of reducing sugar when glucose is used as a standard. In one experiment, an incubation of C. meridionalis style enzyme and glycogen was allowed to proceed for 15 hours and the reducing sugar release was measured by both methods. Using a glucose calibration curve (Table 14), reducing sugar release measured by the Bernfeld method was 0,899 mg glucose.mg protein^{-1}

while that for the Nelson-Somogyi method was 0,460 mg glucose.mg protein^{-1}. Thus the Bernfeld estimation remained approximately 2 times higher than the Nelson-Somogyi estimate, which is similar to differences (approximately 2-2,5) in reducing sugar estimates obtained during 9 minute incubations of style enzymes and a glycogen substrate (Table 12). Therefore increasing the incubation time of substrate and style enzymes from 9 minutes to as much as 15 hours does not reduce the discrepancy between the two methods. However, if maltose had been used as a standard (Table 14), reducing sugar release would be calculated as 1,088 mg maltose.mg protein^{-1} by the Bernfeld method and 1,092 mg maltose.mg protein^{-1} by the Nelson-Somogyi method. This suggests that the final reaction product was a disaccharide for which maltose was a more appropriate standard than glucose.

Glucose is therefore not necessarily the correct reducing sugar to use for calibration curves to quantify molluscan enzyme activity of α-amylase, laminarinase and cellulase. In short incubations, α-amylase hydrolyses starch and glycogen to 70% -90% maltose and small amounts of glucose and dextrins (Diem and Lentner, 1970). Cellulase hydrolyses cellulose to cellobiose units (Hart and Schuetz, 1966), and laminarinase hydrolyses laminarin to laminaribiose, higher oligosaccharides and some glucose but laminaribiose is hydrolysed to glucose very slowly (Chesters and Bull, 1963; Sova et al., 1970). Seiderer et al., (1982) show that

α-amylase and laminarinase account for a very large proportion of glucogenic activity of crystalline styles of the mussels C. meridionalis and P. perna, and other workers show that α-amylase and laminarinase are the principal bivalve carbohydrases (Horiuchi and Lane, 1966; Sova et al., 1970; Kristensen, 1972a). On this basis it would be more accurate to establish calibration curves using a disaccharide reducing sugar.

Recalculation of reducing sugars produced by enzyme activity of mussel styles on various substrates as given in Table 12, are shown in Table 15 using maltose rather than glucose as a standard. Reducing sugars assayed by the Bernfeld method are still higher than the Nelson-Somogyi method, but only by a factor of between 1,08 and 1,37. Clearly, an accurate and consistent measure of reducing sugar release depends upon selecting an appropriate sugar for the calibration curve. The sugar used as the standard should be representative of the dominant end products of substrate hydrolysis.

Implications for quantitative estimates of reducing sugars.

Previous studies involving reducing sugar release by molluscan crystalline style enzymes, estimated by the Bernfeld or Nelson-Somogyi method, have often used calibration curves based on a glucose standard (Sova et al., 1970; Kristensen, 1972a; Lindley et al., 1976; Gianfreda et al., 1979; Seiderer and Newell, 1979; Seiderer et al., 1982; Lucas and Newell, 1984). From these results it is

TABLE 15

Recalculation of reducing sugar release by C. meridionalis style enzyme, using a maltose calibration curve from Table 13. Units are mg maltose.mg protein^{-1}.h^{-1}

Incubation	Enzyme activity		Discrepancy
	Bernfeld method	N-Somogyi method	Bernfeld / N-Somogyi
Style Enzyme + Glycogen	5,73	5,29	1,08
Style Enzyme + Starch	11,82	8,62	1,37
Style Enzyme + Laminarin	0,45	0,38	1,18
Style Enzyme + CMC	0,00	0,19	-

evident that both methods can be used in this way to detect the presence of reducing sugars, although the Nelson-Somogyi method is the more sensitive but provides a lower estimate of reducing sugars with a glucose standard, as well as being less affected by the conditions of the incubation (Miller, 1959; Farouki and Gunn, 1983; Breuil and Saddler, 1985). The selection of glucose as a standard is not unwarranted when comparative or qualitative results are sought. However, the reducing sugars present after short incubations of polysaccharide substrates with extracts of digestive gland or crystalline style enzymes are likely to be mainly disaccharides, and the molecular weight of the reducing sugar used as a standard has a marked effect on the calibration curve. It is therefore important to select an appropriate standard when making quantitative estimates of sugar release, particularly when those results are used to calculate energy budgets. For this purpose it is neccessary to know what the reaction products of an enzyme-substrate incubation are likely to be before selecting a single reducing sugar as a standard for calibration purposes.

To test the significance of these findings, the energy budgets of Seiderer et al., (1982) and Lucas and Newell (1984) may be recalculated using more appropriate maltose and cellobiose standard regressions from Table 14, since both authors use glucose only as a standard. It makes very little difference to recalculate the Bernfeld assays of Seiderer et al., (1982) in terms of maltose equivalents since their

standard (glucose) to make quantitative estimates of reducing sugar release, in an effort to calculate the energetic gain from crystalline style activity, it seems likely that such energy balance studies may be in error.

CHAPTER IV

α-AMYLASE AND LAMINARINASE ACTIVITIES IN THE STYLE AND DIGESTIVE GLAND OF FOUR SOUTH AFRICAN MUSSELS

INTRODUCTION

Although a considerable body of literature exists on digestive enzymes in marine invertebrates, very little of it directly concerns the ecological implications of enzyme activity. The relationship between the spectra of carbohydrases and the natural food sources of these animals has been examined but the presence of laminarinase and cellulase enzymes is likely to be more closely correlated with phylogenetic relationships than with diet (Sova et al., 1970; Gianfreda et al., 1979; Agnisola et al., 1981). Most analyses of enzyme activities in bivalves have been confined to establishing their presence, temperature and pH optima, and the conditions suitable for hydrolysis. In general α-amylase and laminarinase appear to be the most important polysaccharases in bivalves (Wojtowicz, 1972; Shallenberger et al., 1974; Seiderer et al., 1982; Stark and Walker, 1983).

Polysaccharide hydrolase studies can however supply useful information about the ecology of a particular animal, as shown by the work of Seiderer and Newell, (1979), Seiderer et al., (1982) and Lucas and Newell, (1984). Thus although the carbohydrase spectra of marine invertebrates may not be closely correlated with food sources, in animals sharing the same food sources, enzyme activities may be a mechanism controlling the distribution and ecological success of species under different environmental conditions. The ability to digest the available food source in order to acquire

suffficient energy from the environment will clearly be a major factor determining the success of a species in a particular habitat.

Apart from the work of Seiderer and Newell (1979) and Seiderer et al. (1982) on C. meridionalis, the only other information on polysaccharide carbohydrases in South African mussels is that of Gardiner (1980). He recorded no increase in the crystalline style α-amylase activity of subtidal A. ater, after warm acclimation. To date there is no information on digestive gland carbohydrases of these mussels. If the digestive gland significantly contributes to saccharogenesis during digestion, the saccharogenesis required to satisfy the animals carbon requirements from crystalline style enzymes would be considerably less. Style turnover times such as those determined by Seiderer et al. (1982) may therefore be longer. The following work was carried out to compare the major crystalline style and digestive gland carbohydrases of C. meridionalis, M. galloprovincialis , P. perna and A. ater and to examine these enzyme activities in relation to the habitat and distribution of the different species. The hypothesis is tested that the differing mussel distributions on the South African coastline can in part be explained by differing enzyme capabilities.

MATERIALS AND METHODS

Preliminary work on the crystalline style and digestive gland enzymes of C. meridionalis, M. galloprovincialis, P. perna and A. ater indicated that α-amylase and laminarinase were the major carbohydrases, and cellulase and alginate lyase accounted for less than 1% of total saccharogenesis. Experiments were therefore confined to testing the effect of the two major polysaccharases on commercially available and purified substrates.

Experimental material

C. meridionalis, M. galloprovincialis and A. ater of approximately 60 mm shell length were collected from the intertidal rocks at Blouberg Strand on the west coast of South Africa, while P. perna ($\pm$ 60 mm shell length) were collected intertidally at Bailey's Cottage in False Bay (Figure 1). Enzyme solutions were prepared from crystalline style and digestive gland material immediately on returning to the laboratory, all assays being completed within 24 hours of collection. Material for rate-temperature and gel filtration experiments was collected on separate occasions.

The crystalline style and approximately 400 mg wet weight of digestive gland tissue from the area immediately surrounding the stomach were removed from each of 15 individuals of each species. Tissues were rinsed in chilled 20 mM phosphate buffer pH 6,9 containing 150 mM NaCl, and homogenized with a

glass tissue grinder in 5 ml of 20 mM chilled phosphate buffer (see also Seiderer et al., 1982; Lucas and Newell, 1984). Crystalline style homogenates were centrifuged at 15000 x g at 3^0C for 10 minutes, while digestive gland homogenates were centrifuged at 30000 x g at 3^0C for 60 minutes. The supernatant was used for enzyme assays. The supernatant from digestive gland samples was filtered through a glass wool filter to remove any undissolved lipids. As preliminary work indicated high enzyme blanks in digestive gland preparations, these were dialysed at 3^oC for approximately 20 hours against 3000 volumes of stirred 20 mM phosphate buffer.

Style and digestive gland protein concentrations

Protein concentration of the enzyme preparations was calculated using the method of Lowry et al. (1951), from the standard equation described by $y = 0{,}025 + 0{,}00097x$ ($r = 0{,}99$, $n = 8$) where y is the absorbance at 660 nm and x is the protein concentration in $\mu g.ml^{-1}$, with bovine serum albumin (BDH) as a standard.

Effect of temperature on enzyme activity.

Enzyme preparations were all standardized to 1 mg protein.ml^{-1} and α-amylase and laminarinase activities were determined by the method of Nelson (1944) as modified by Somogyi (1951), using commercially available and purified oyster glycogen and laminarin substrates as described in

Chapter III. However, 200 µl aliquots of enzyme and substrate were used instead of 500 µl aliquots. After incubating at each temperature for 5 minutes, 400 µl of Somogyi reagent was added. After boiling and cooling, 800 µl of Nelson reagent and 5 ml of water were added. The absorbance at 660 nm was determined using a Beckman DU 40 Spectrophotometer, and saccharogenic activity was calculated from the standard calibration equation $y = -0,02 + 1,77x$ ($r = 1$, $n = 7$) where y is the absorbance at 660 nm and x is reducing sugar concentration in $mg.ml^{-1}$, using maltose as a standard. Substrate and enzyme blanks were subtracted from experimental values and data points given in the results are the mean of triplicate readings.

Separation of proteins by gel filtration

Aliquots of 3 ml of enzyme extracts prepared as described above, and containing approximately 20 mg protein, were loaded onto an 84 cm x 2 cm diameter gel filtration column packed with Sephacryl S-200. The sample was eluted with approximately 300 ml of 20 mM phosphate buffer. However, no further proteins were detected in fractions after the elution of approximately 200 ml of buffer. The eluate was collected in 2 ml fractions using a fraction collector. Because of the large number of samples involved, the protein in each fraction was determined spectrophotometrically at 280 nm from the standard equation $y = 0,003 + 0,00067x$ ($r = 0,99$, $n = 8$) where y is the absorbance at 280 nm and x is the protein concentration in $\mu g.ml^{-1}$, using bovine serum albumin as a

standard. However, it was found that protein concentrations determined by this method overestimated the concentration when compared with estimates based on the method of Lowry et al., (1951). Thus protein concentrations of 53 enzyme fractions were measured by both methods, and protein concentrations determined at 280 nm were corrected to protein concentrations measured at 660 nm (Lowry et al., 1951) using the equation $y = 1,67 + 1,85x$; ($r = 0,97$, $n = 53$) where y is the protein concentration at 280 nm and x is the protein concentration at 660 nm. α-amylase and laminarinase activity in alternate fractions was determined as described above.

The approximate molecular weight of proteins eluted from the column was estimated from the elution volume using bovine serum albumin, ovalbumin, lysozyme and cytochrome C as standards. These standards (2 mg) were eluted from the column with approximately 300 ml of 20 mM phosphate buffer and the fractions were scanned at 280 nm to locate the protein peaks. The elution volume of the protein standards was then used to construct the calibration curve $y = 1225 - 235,28 \text{ Log } x$ ($r = -0,98$, $n = 4$) where y is the elution volume in ml and x is the molecular weight.

RESULTS

Rate-temperature curves

Rate-temperature curves of the crystalline style and digestive gland α-amylase and laminarinase activities of C. meridionalis, M. galloprovincialis, P. perna and A. ater are shown in Figures 23-26. The temperature coefficients of these curves are shown in Table 16.

a) α-amylase activity

Between 10°C and 20°C the crystalline style α-amylase activity Q_{10}s of all the mussels were very similar, ranging between 1,99 and 2,18 (Figure 23, Table 16). These Q_{10} values thus approach the Q_{10} for bivalve filtration rates in this temperature range (Winter, 1978). Since water temperatures in False Bay (Cliff, 1979) and on the Atlantic coast (see Chapter I) are between 10°C and 20°C most of the time, a considerable increase in digestion and assimilation is possible as a result of greater α-amylase activities following rapidly increasing environmental temperatures. This would allow the animals to benefit from temperature-induced increases in clearance rates and help to offset the increased metabolic cost associated with higher temperatures (Bayne and Newell, 1983). Temperature coefficients for style α-amylase activity between 20°C and 30°C were much lower than those between 10°C and 20°C (see Table 16,) indicating an adaptation to the lower temperature range. However, it is

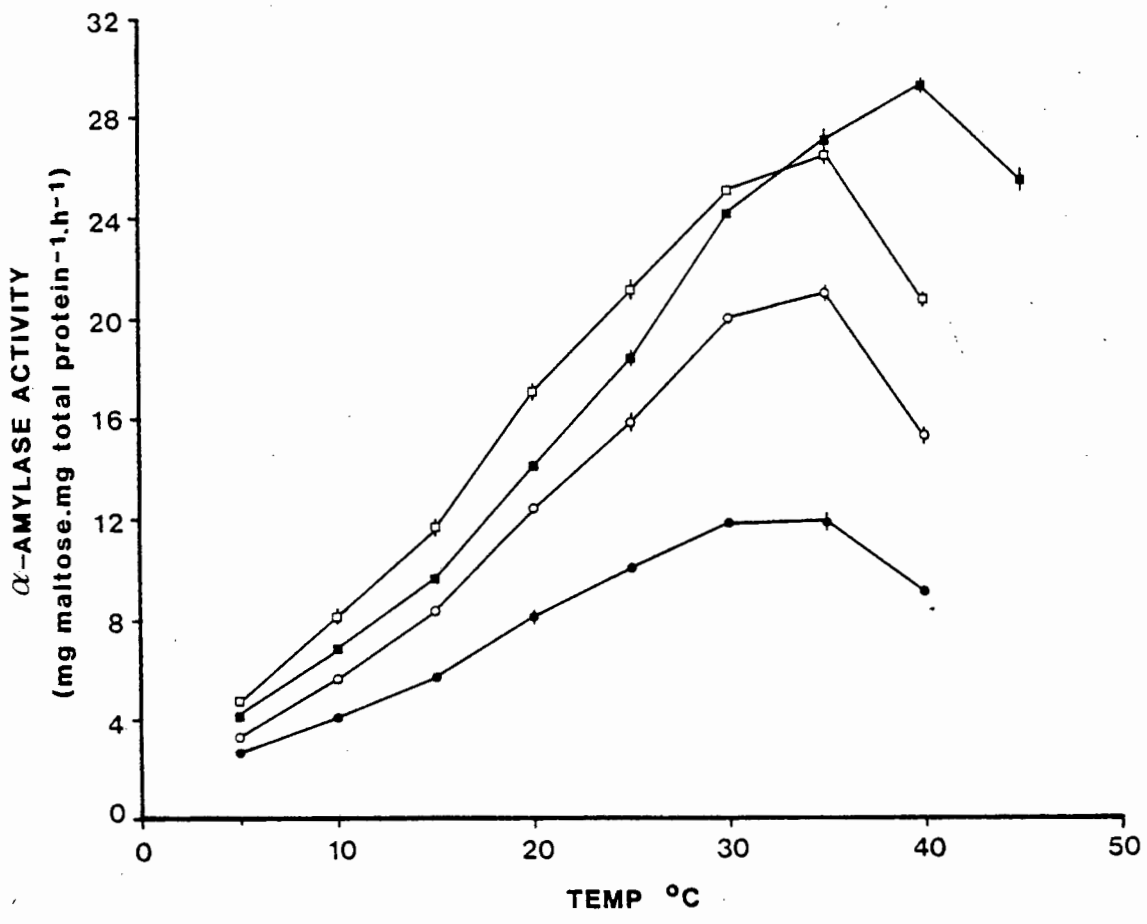


Figure 23. Crystalline style α-amylase rate-temperature curves for C. meridionalis (●—●), M. galloprovincialis (□—□), P. perna (■—■) and A. ater (o—o). Vertical bars represent the standard deviation of the mean.

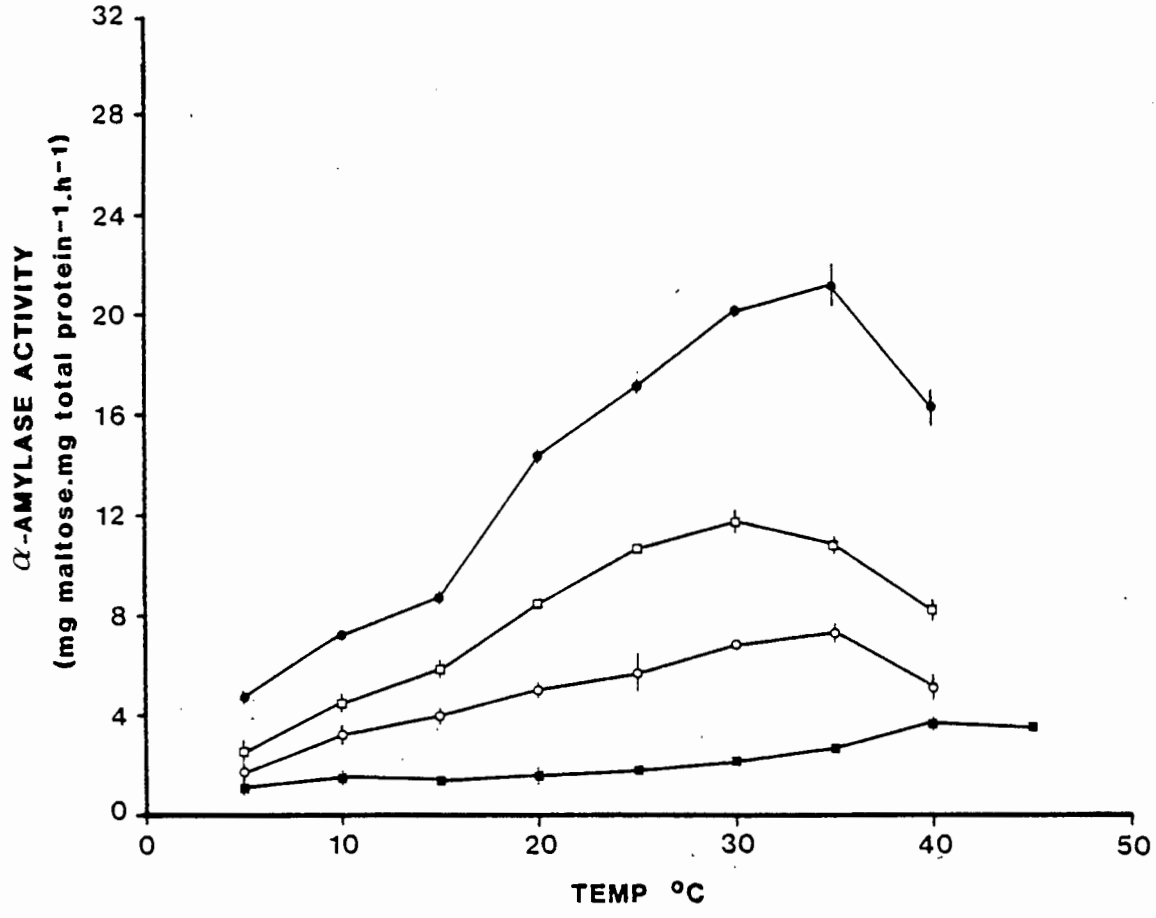


Figure 24. Digestive gland α-amylase rate-temperature curves for C. meridionalis (●—●), M. galloprovincialis (□—□), P. perna (■—■) and A. ater (○—○). Vertical bars represent the standard deviation of the mean.

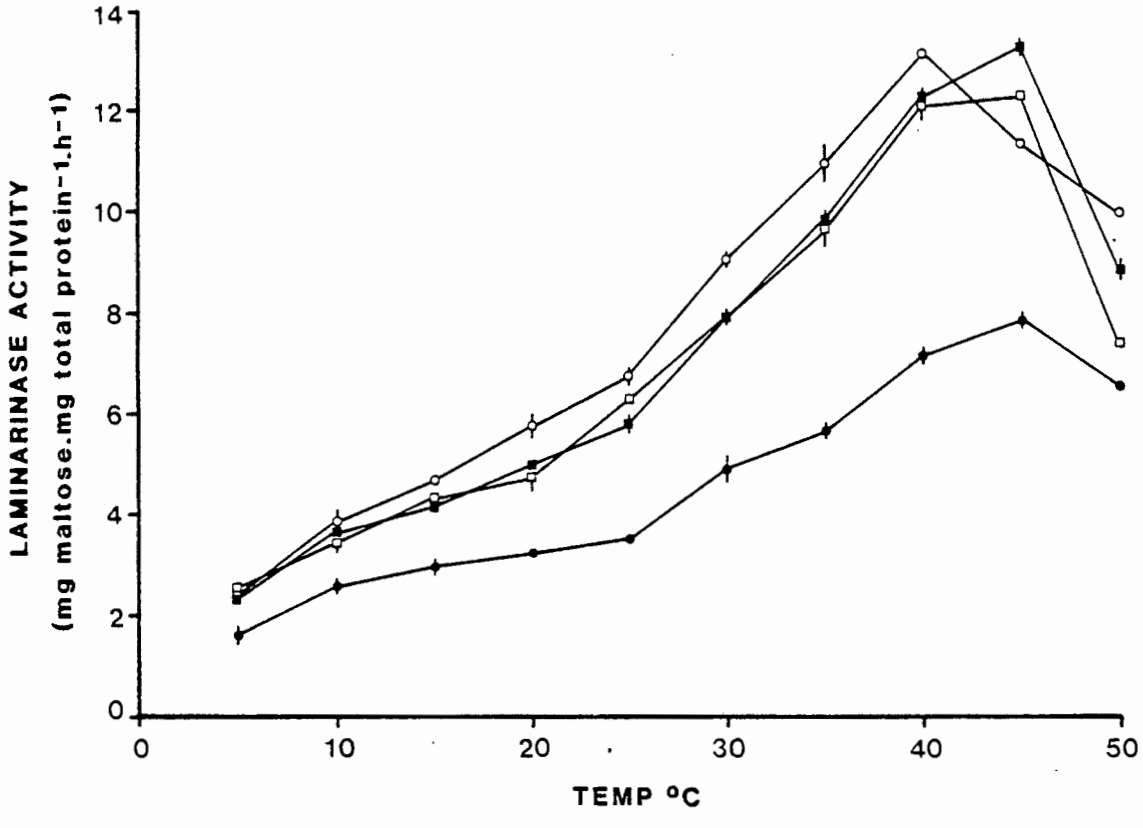


Figure 25. Crystalline style laminarinase activity rate-temperature curves for C. meridionalis (●—●), M. galloprovincialis (□—□), P. perna (■—■) and A. ater (○—○). Vertical bars represent the standard deviation of the mean.

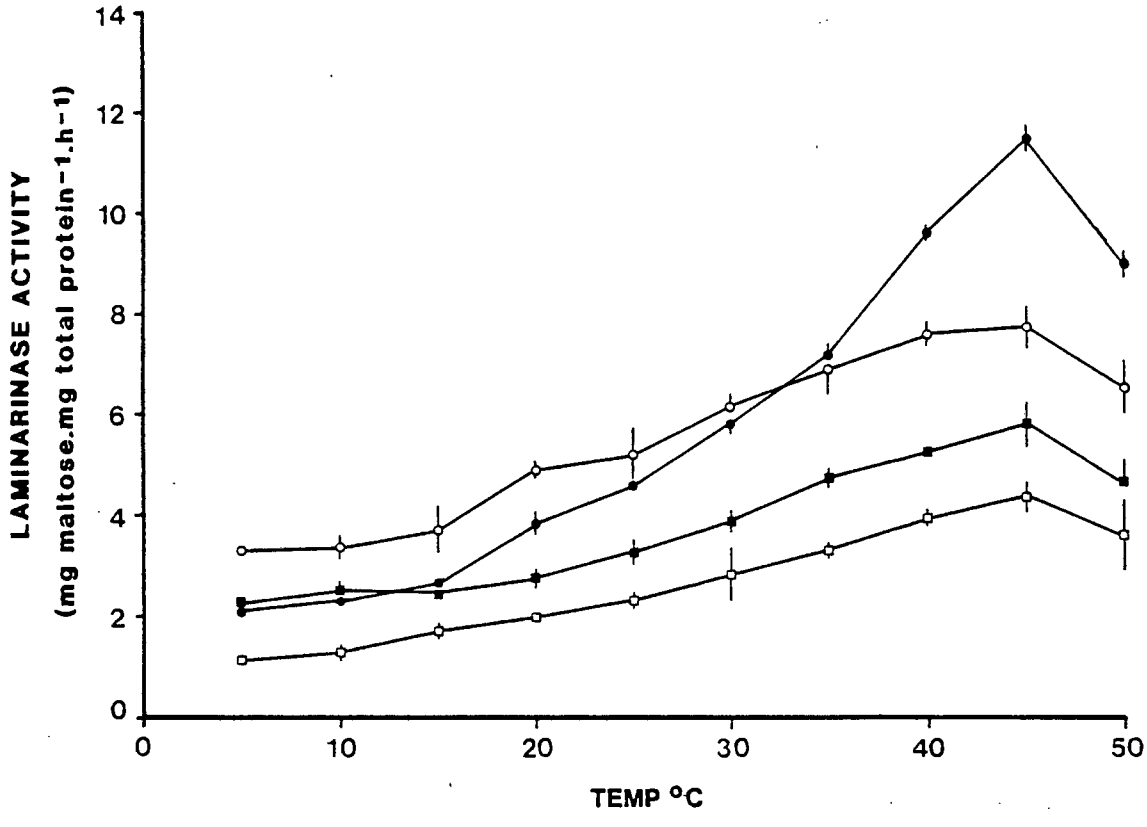


Figure 26. Digestive gland laminarinase activity rate-temperature curves for C. meridionalis (●—●), M. galloprovincialis (□—□), P. perna (■—■) and A. ater (o—o). Vertical bars represent the standard deviation of the mean.

TABLE 16

Temperature coefficients for crystalline style and digestive gland α-amylase and laminarinase acitivity of four mytilid species.

Mussel	Style				Digestive gland			
	α-amylase		laminarinase		α-amylase		laminarinase	
	10-20°C	20-30°C	10-20°C	20-30°C	10-20°C	20-30°C	10-20°C	20-30°C
C. meridionalis	1,99	1,45	1,25	1,52	1,97	1,40	1,67	1,53
M. galloprovincialis	2,11	1,47	1,37	1,65	1,99	1,38	1,47	1,42
P. perna	2,06	1,72	1,36	1,61	1,00	1,42	1,13	1,41
A. ater	2,18	1,60	1,45	1,63	1,51	1,37	1,48	1,25

interesting that the Q_{10} between 20°C and 30°C for P. perna, which is generally confined to the warmer south and east coasts, was higher (1,72) than for any of the cold west coast species (1,45-1,60).

Digestive gland α-amylase rate-temperature curves and temperature coefficients shown in Figure 24 and Table 16 demonstrated a wide variation. Between 10°C and 20°C C. meridionalis and M. galloprovincialis had high Q_{10}s in this range (1,97 and 1,99 respectively), which are very similar to these species' Q_{10} values for crystalline style α-amylase activity (Table 16). A. ater had a digestive gland α-amylase activity Q_{10} of 1,51 between 10°C and 20°C compared with a crystalline style coefficient of 2,18 in this temperature range. However, P. perna had a digestive gland α-amylase temperature coefficient of 1,00 in the 10°C-20°C temperature range, and Figure 24 shows the extremely flat nature of the rate-temperature curve, and the low level of saccharogenesis. This is surprising and indicates that P. perna relies almost exclusively on energy production by the crystalline style to combat increased metabolic costs associated with raised temperatures.

The optimum temperature for crystalline style α-amylase activity was 40°C for P. perna from the warm south coast, and 35°C for the three west coast cold water species (Figure 23). Digestive gland α-amylases show the same pattern except that the optimum temperature for M. galloprovincialis was 30°C

(Figure 24) and this species may therefore possess two isozymes of α-amylase with differing temperature optima (Alemany and Rosell-Perez, 1973; Mirza and Serban, 1981). The higher temperature optima for P. perna α-amylases may allow this species to penetrate further into the progressively warmer climatic conditions along the south and east coasts than the other species. However, the elevated temperature optima may not be inherent and could also be a response to water temperatures that in summer are generally 5-6^{0}C warmer in False Bay than the Atlantic coast.

b) Laminarinase activity

Changes in crystalline style laminarinase activity with temperature (Figure 25) were very similar for all mussel species and Q_{10} values between 10^{0}C and 20^{0}C ranged between 1,25 and 1,45 (Table 16). A. ater which is predominantly a kelp bed animal had the highest coefficient for style laminarinase activity. However these values were considerably lower than α-amylase coefficients in this temperature range (1,99-2,18). Temperature coefficients in the 20^{0}C-30^{0}C temperature range were similar to crystalline style α-amylase Q_{10}s between these temperatures (Table 16).

Digestive gland laminarinase rate-temperature curves of the mussels are shown in Figure 26 and the temperature coefficients are shown in Table 16. Q_{10} values between 10^{0}C and 20^{0}C for C. meridionalis, M. galloprovincialis and A. ater were similar (1,47-1,67) and once again were higher

than corresponding Q_{10} values between $20^{o}C$ and $30^{o}C$. P. perna had a very low Q_{10} (1,13) between $10^{o}C$ and $20^{o}C$ and a higher value (1,41) for the $20^{o}C$ to $30^{o}C$ temperature range.

For C. meridionalis, M. galloprovincialis and P. perna, the optimum temperature for crystalline style and digestive gland laminarinase activity was $45^{o}C$. For A. ater however, the optimum temperature for style laminarinase activity was $40^{o}C$ while that for digestive gland laminarinase activity was $45^{o}C$. There may therefore be two types of laminarinase isozymes present in the digestive gland and crystalline style tissues of A. ater (Figures 25 and 26).

In Figures 23 and 25 it is evident that α-amylase and laminarinase activity per unit protein in crystalline style homogenates was lower at all temperatures for C. meridionalis than for M. galloprovincialis, P. perna and A. ater. This is probably the result of a greater proportion of structural protein in the crystalline style of C. meridionalis than in the styles of M. galloprovincialis, P. perna and A. ater. The dry weight of crystalline style as a percentage of dry body weight of 10 animals of each species was measured and is shown in Table 17. C. meridionalis had a greater weight of style per unit flesh weight than the other species and the increasing α-amylase activity per unit protein of the different species shown in Figure 23 was matched by a decrease in style weight as a percentage of body weight (Table 17). In digestive gland material however, the

TABLE 17

Dry weight of the crystalline style as a percentage of dry body weight in *C. meridionalis*, *M. galloprovincialis*, *P. perna* and *A. ater*.

Mussel	Style wt. as a percentage of body weight (± SD)	
C. meridionalis	0,460	(± 0,054)
M. galloprovincialis	0,134	(± 0,097)
P. perna	0,146	(± 0,076)
A. ater	0,245	(± 0,080)

α-amylase activity per unit protein of C. meridionalis was considerably higher than that of the other species (Figure 24).

Gel filtration of enzyme proteins

a) Protein profiles

The recovery of style and digestive gland proteins loaded onto the column is shown in Table 18, and ranged between 52% and 76%. The recovery of style protein was generally greater (71%) than digestive gland protein (58%). Protein distributions in relation to elution volumes for crystalline style and digestive gland preparations of the four mussel species are shown in Figure 27. Although all digestive gland protein profiles and all crystalline style protein profiles were fairly similar, considerable differences were evident between style and digestive gland profiles. Style proteins showed a dominant peak at an elution volume of 62 ml which, from the protein calibration standards, corresponds to a molecular weight of approximately 88000. There was a further broad protein peak or shoulder at an elution volume of approximately 110 ml (MW $\simeq$ 55000) and a third peak corresponding to proteins with molecular weights < 44000.

Digestive gland protein profiles were dominated by the elution of high molecular weight proteins (> 50000) between elution volumes of approximately 55 ml and 120 ml. Thereafter M. galloprovincialis and A. ater showed a gradual decrease in

TABLE 18

Percentage recovery of crystalline style and digestive gland proteins of C. meridionalis, M. galloprovincialis, P. perna and A. ater after gel filtration.

Mussel	Percentage Recovery	
	crystalline style	digestive gland
C. meridionalis	76%	62%
M. galloprovincialis	74%	64%
P. perna	64%	53%
A. ater	71%	52%
Mean	71%	58%

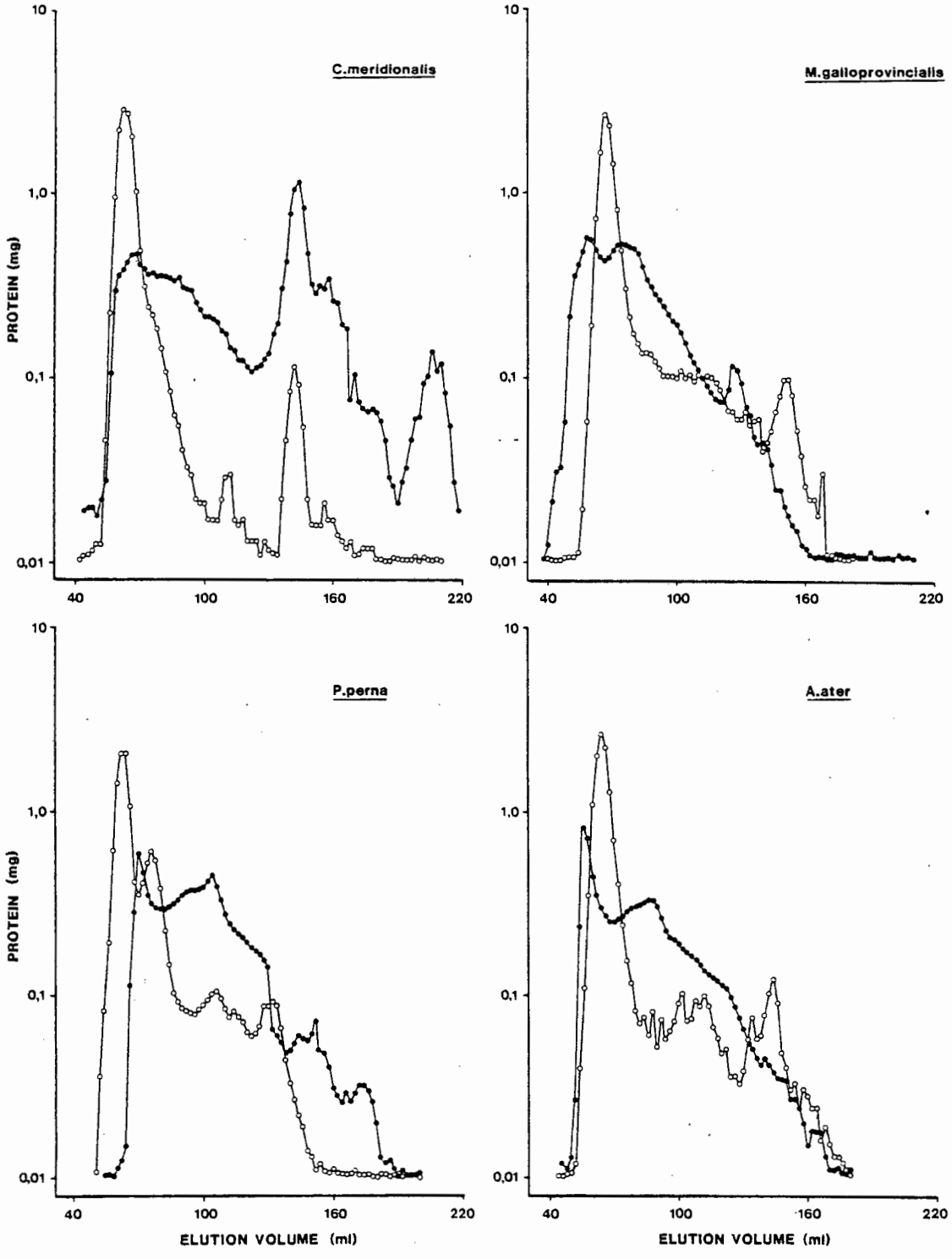


Figure 27. Protein weight (mg) as a function of elution volume in style (o——o) and digestive gland (•——•) homogenates of C. meridionalis, M. galloprovincialis, P. perna and A. ater. Data expressed for the addition of 20 mg protein to the column.

protein content of the eluate fractions while C. meridionalis and P. perna had well defined peaks corresponding with proteins of molecular weights < 37000.

After a single gel filtration, enzyme proteins cannot be completely separated from structural proteins. This results in a supression of enzyme activity per unit protein in larger molecular weight fractions which may contain structural material. However, smaller molecular weight fractions may approach the specific activities of the enzymes (Seiderer et al., 1982). Thus estimates of saccharogenesis by α-amylase and laminarinase were expressed per unit of total protein in the eluate.

b) α-amylase activity

The profile of α-amylase activity in C. meridionalis, M. galloprovincialis, P. perna and A. ater crystalline style and digestive gland eluates is shown in Figure 28, where α-amylase activity is plotted as a function of elution volume. "Specific activities" of the enzyme peaks together with elution volumes are shown in Table 19. There were considerable differences in α-amylase activity profiles, both among the different mussel species and between digestive gland and style material. All four mussel species show similar crystalline style α-amylase activity peaks at an elution volume of 62 ml (MW $\simeq$ 88000), which coincides with the large molecular weight structural proteins in the style (Figure 27). C. meridionalis and P. perna each have one other

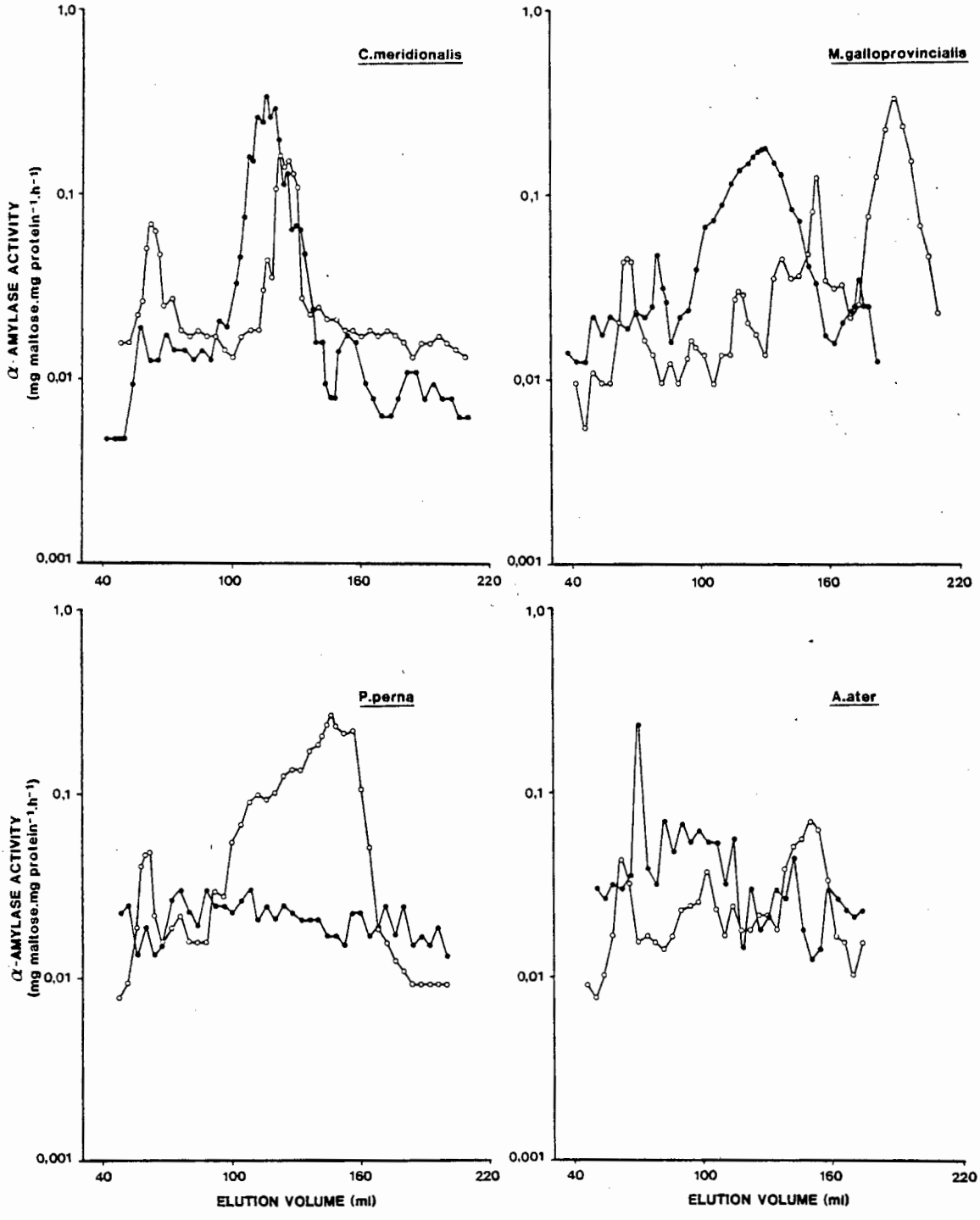


Figure 28. Saccharogenic activity (mg maltose liberated. mg protein in eluate^{-1}.h^{-1} at 25° C) of style (o——o) and digestive gland (•——•) α-amylases of C. meridionalis, M. galloprovincialis, P. perna and A. ater expressed as a function of elution volume (ml).

TABLE 19

Specific activities, elution volumes and approximate molecular weights of C. meridionalis M. galloprovincialis, P. perna and A. ater crystalline style and digestive gland α-amylase proteins Enzyme activity is expressed as mg maltose liberated at 25°C.mg protein^{-1} in the eluate^{-1}.h

Mussel	Crystalline style			Digestive gland		
	Elution vol ml	Approx. MW	Activity mg maltose. mg protein^{-1}.h^{-1}	Elution vol ml	Approx. MW	Activity mg maltose. mg protein^{-1}.h^{-1}
C. meridionalis	62	88000	0,74	116	52000	70,81
	124	48000	341,90			
M. galloprovincialis	62	88000	1,06	80	74000	48,73
	118	51000	12,55	± 130	45000	2,35
	154	36000	59,78	174	29000	257,30
	190	25000	3142,21			
P. perna	62	88000	1,25	-		
	146	39000	972,91	-		
A. ater	62	88000	0,67	70-114	-	1,27-6,33
	102	59000	11,55	142	40000	14,45
	150	37000	52,72			

well defined peak corresponding to a molecular weight of < 48000, while A. ater had two and M. galloprovincialis three further α-amylase activity peaks (Table 19). For M. galloprovincialis, the peak at 190 ml was the largest and had a "specific activity" of 3142,21 mg maltose.mg protein^{-1}.h^{-1}, which is very high compared with other α-amylase "specific activities" shown in Table 19. Although the estimates of the molecular weights of enzyme peaks differ, these style α-amylase protein profiles for C. meridionalis and P. perna agree well with those of Seiderer et al. (1982). In all eluates the "specific activity" of the first peak is probably reduced because of structural proteins present in the eluate, but lower molecular weight peaks can reach very high apparent "specific activities", particularly in M. galloprovincialis and P. perna style eluates (see Table 19).

In digestive gland α-amylase activity profiles, there was little α-amylase activity in the large molecular weight structural proteins which eluted first from the column, apart from a possible peak denoted by a single point at an elution volume of 70 ml in the A. ater profile (Figure 28). C. meridionalis had a single large well defined α-amylase protein peak and M. galloprovincialis had a large broad peak and two smaller peaks (see Figure 28, Table 19). In A. ater eluates, α-amylase protein peaks were poorly defined, but there is considerable α-amylase activity between elution volumes of 70 ml and 114 ml. There were no clearly defined

peaks in α-amylase activity of P. perna digestive gland proteins and α-amylase activity was very low. This confirms the low saccharogenic rate for this species shown in Figure 24. "Specific activities" of C. meridionalis and M. galloprovincialis digestive gland α-amylase were considerably higher than those of P. perna and A. ater (Table 19) and this agreed well with saccharogenesis per unit total protein shown in Figure 24. In digestive gland eluates M. galloprovincialis again had an α-amylase activity peak (MW $\approx$ 29000) with a very much greater "specific activity" than any of the other mussel species (Table 19). From Figure 28 it would appear that in M. galloprovincialis and C. meridionalis there is approximately equal α-amylase activity in the crystalline style and digestive gland, while for P. perna, greater α-amylase activity is located in the style than the digestive gland, while the reverse is true for A. ater.

c) Laminarinase activity

Profiles of the laminarinase activity of the crystalline style and digestive gland proteins of the four mussel species are shown in Figure 29 and "specific activities"of the enzyme peaks are shown in Table 20. In crystalline style eluates, all four species had a well defined laminarinase activity peak at an elution volume of 62 ml (MW $\approx$ 88000). Style eluates showed a second peak for C. meridionalis and two others for P. perna and M. galloprovincialis. Seiderer et al. (1982) found 2 laminarinase protein peaks in crystalline style eluates of C. meridionalis and P. perna, and again

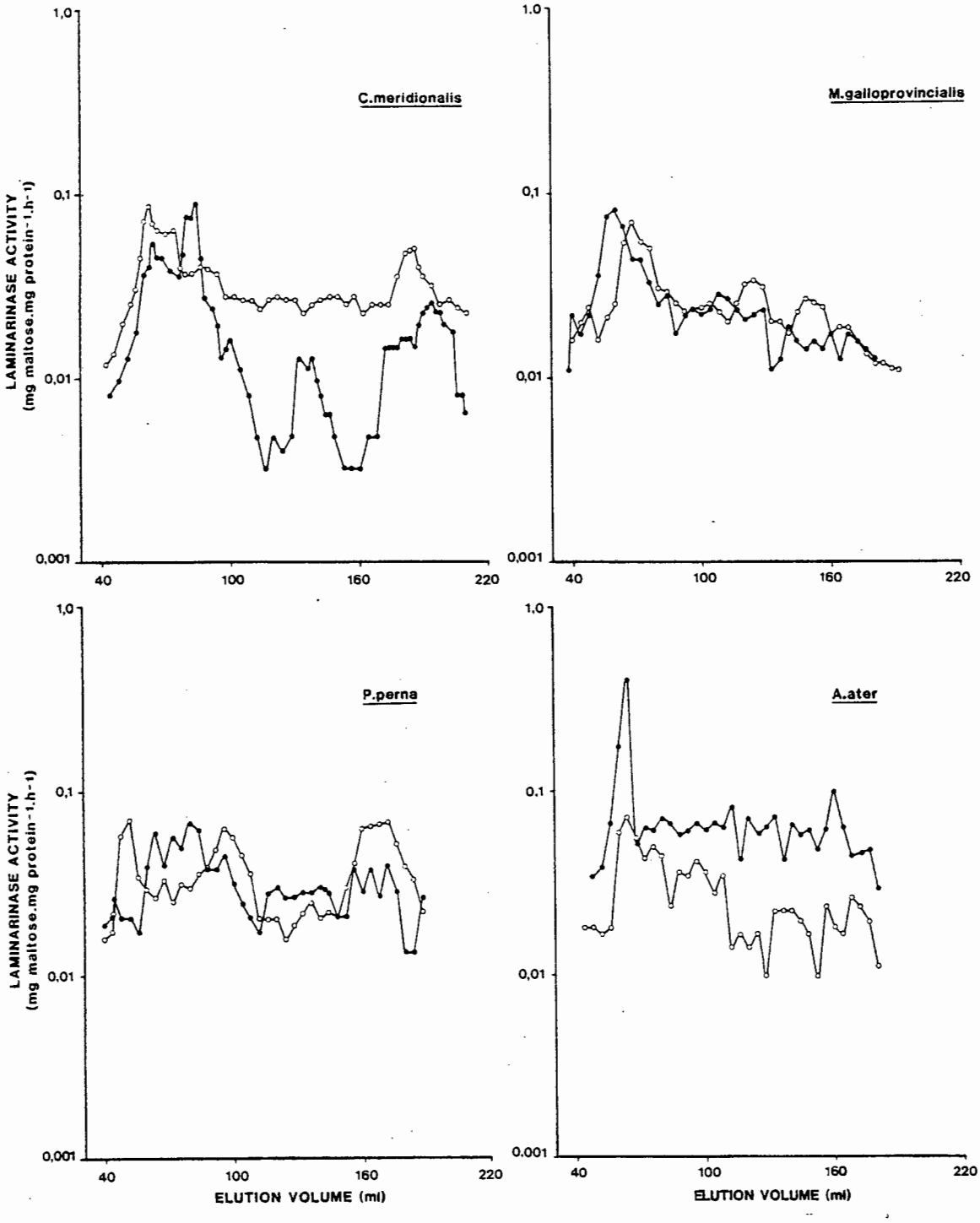


Figure 29. Saccharogenic activity (mg maltose liberated. mg protein in eluate.h^{-1} at 25^{o} C) of style (o——o) and digestive gland (•——•) laminarinases of C. meridionalis, M. galloprovincialis, P. perna and A. ater expressed as a function of elution volume (ml).

TABLE 20

Specific activities, elution volumes and approximate molecular weights of C. meridionalis, M. galloprovincialis, P. perna and A. ater crystalline style and digestive gland laminarinase proteins. Enzyme activity is expressed as mg maltose liberated at 25°C.mg protein in the eluate^{-1}.h^{-1}.

Mussel	Crystalline style			Digestive gland		
	Elution vol ml	Approx. MW	Activity mg maltose. mg protein^{-1}.h^{-1}	Elution vol ml	Approx. MW	Activity mg maltose. mg protein^{-1}.h^{-1}
C. meridionalis	62	88000	0,91	60-90	-	2,66-2,05
	186	26000	686,87	138	42000	12,56
				194	24000	20,90
M. galloprovincialis	62	88000	5,18	60-90	-	3,70-2,08
	124	48000	19,75	108	56000	5,75
	150	37000	19,03			
P. perna	62	88000	2,17	60-90	-	0,76-1,14
	106	57000	15,28	140	41000	5,45
	178	28000	460,50	174	29000	50,79
A. ater	62-104	-	1,57-11,11	64	86000	16,71
	136	43000	10,69			
	168	31000	39,15			

their estimates of the molecular weights of the protein peaks differ from those reported here (Table 20).

In A. ater, style proteins showed elevated laminarinase activity in the elution range 62 ml-104 ml and in two smaller broad peaks. Thus M. galloprovincialis, P. perna and A. ater each have three laminarinase protein peaks while C. meridionalis has two (Figure 29, Table 20). Figure 25 shows that M. galloprovincialis, P. perna and A. ater had very similar laminarinase activities per unit total style protein, while that of C. meridionalis was considerably lower. However, Table 20 shows that the "specific activities" of crystalline style laminarinases of C. meridionalis and P. perna are very much higher than those of M. galloprovincialis and A. ater.

In contrast to α-amylase proteins, laminarinase proteins were located in the large molecular weight digestive gland proteins which eluted early from the column (Figure 29). "Specific activities" of these laminarinase proteins are shown in Table 20. The elution profile of A. ater indicated a raised level of digestive gland laminarinase activity throughout the range of proteins eluted. Figure 26 showed that below 33^{0}C, saccharogenesis by A. ater digestive gland laminarinase per unit total protein was higher than the other mussels. C. meridionalis, M. galloprovincialis and P. perna all had broad digestive gland laminarinase protein peaks between elution volumes of approximately 60 ml and 90 ml, and

either one or two minor peaks (Figure 29, Table 20). In all mussel species, the "specific activities" of digestive gland laminarinases were generally lower than those of style laminarinases (Table 20), but this may be a result of the high levels of protein that occurred throughout much of the elutions of digestive gland homogenates (Figure 27). Laminarinase activity appears to be equally distributed between the crystalline style and digestive gland in M. galloprovincialis and P. perna, while more activity is found in the style in C. meridionalis and in the digestive gland in A. ater (Figure 29). It is noticeable that in these last two species, which are particularly associated with the kelp beds, laminarinase activity in the style (C. meridionalis) and digestive gland (A. ater) is high throughout the elution range.

DISCUSSION

Rate-temperature curves

The temperature coefficients for crystalline style α-amylase activity in Table 16 are very much higher than the values of 1,21 reported for C. meridionalis style α-amylase activity by Seiderer and Newell (1979) and 1,20 for subtidal A. ater style α-amylase activity by Gardiner (1980). The reason for this is not clear, but it is possible that mussels are sensitive to the amplitude of temperature variations

encountered in the intertidal area, and during periods of large temperature fluctuations, can increase the temperature coefficients of the style enzymes. Seiderer and Newell (1979) used C. meridionalis collected from Blouberg Strand in winter (Seiderer, pers comm.) while Gardiner used subtidal A. ater. In both cases the temperature fluctuations experienced by the animals were very much less than the mid-summer conditions experienced by animals in the present study. In summer, submerged mussels experience temperatures of 10-12^{0}C and on exposure temperatures may be as high as 30^{0}C. Thus apart from the thermal acclimation response in C. meridionalis style α-amylase shown by Seiderer and Newell (1979), there may be a seasonal increase in α-amylase temperature coefficients in response to the large daily fluctuations in temperature that the animals are subjected to in summer. Such seasonal changes may be fairly common, since Gabbott (1983) describes seasonal changes in various enzyme activities of a number of bivalves (see also Hoffmann, 1983).

There is also a different interpretation of the significance of the acclimatory response in style α-amylase activity of C. meridionalis (Seiderer and Newell, 1979). In summer on the west coast of South Africa, sea temperatures are lower ($\pm$ 12^{0}C) than in winter ($\pm$ 15^{0}C) because of upwelling events (Chapter I). C. meridionalis lives in the low intertidal zone and grows in dense beds up to 0,5 m above low water spring tide levels (Griffiths 1981). Using South African Navy hydrographic tables to convert this figure to metres above

chart datum, on an average day in summer, C. meridionalis 0,5 m above low water spring tide level are submerged for 59,8% and exposed for 40,2% of a 24 hour period. If only daylight hours are considered to result in elevated temperatures being experienced at low tide, mussels at the top of the intertidal range will still be submerged for 56,4% of the time. These figures assume calm sea conditions, which seldom occur on the exposed west coast. Mussels are therefore probably in equilibrium with low sea temperatures for more than 60% of the time in summer. It is difficult to accept that the animals can therefore acclimate to offset increased metabolic costs incurred during exposure in summer. Acclimation and increased α-amylase activity are more likely to occur in response to warmer water conditions in winter. This may serve to increase assimilation of the higher particulate carbon and nitrogen levels in winter (Chapter I) in order to maximize energy gain before the spring spawning period (Griffiths, 1977). Thus mussels may demonstrate considerable flexibility in rearranging enzyme activities to suit particular environmental circumstances.

Table 16 shows that crystalline style α-amylase Q_{10} values between 10^0C and 20^0C of all mussel species were considerably higher than laminarinase values. Except for P. perna this pattern is repeated in digestive gland α-amylases and laminarinases. Thus to increase assimilation rates to counter increased metabolic costs caused by short term temperature increases, the mussels would appear to rely on

saccharogenesis by α-amylase rather than laminarinase enzymes. Also, because the temperature coefficients for α-amylase are higher than for laminarinase, as temperature increases, saccharogenesis by α-amylase will form an increasingly greater proportion of total saccharogenesis by the two enzymes. This is a metabolic advantage, because starch or glycogen is probably hydrolysed more easily than laminarin. There is considerable evidence that hydrolysis of laminarin requires a suite of enzymes consisting of an exo β-D(1-3) glucanase and one or more endo β-D(1-3) glucanases (Chesters and Bull, 1963; Sova et al., 1970; Lindley et al., 1976; Stark and Walker, 1983; Schevchenko, 1986). Although glucose may be one of the products of laminarin hydrolysis (Chesters and Bull 1963; Lindley et al., 1976; Bezukladnikov and Elyakova, 1986) the major products of hydrolysis of laminarin by marine molluscs are oligosaccharides with traces of laminarisaccharides (Bezukladnikov and Elyakova, 1986; Shevchenko et al., 1986). The further action of a broadly specific β-glucosidase is required to complete hydrolysis to the point where the animal can make use of the product (Chesters and Bull, 1963; Sova et al., 1970; Stark and Walker, 1983). There is also evidence that the initial oligosaccharides produced have an inhibiting effect on the hydrolosis of laminarin (Shevchenko et al., 1986). Thus laminarin digestion is likely to be slow. Hydrolysis of starch or glycogen is rapid, the end products being maltose and glucose (Diem and Lentner, 1970; Stark and Walker, 1983). Several molluscs possess a maltase enzyme to rapidly degrade

the maltose to metabolically useful glucose (Horiuchi and Lane, 1965; Stark and Walker, 1983; Mayasich and Smucker, 1986). Thus as a response to rapid increases in metabolic costs, it is more useful to increase hydrolysis by α-amylase rather than by laminarinase enzymes.

The temperature optima for all the enzymes investigated (Figures 23-26) appear unrealistically high for intertidal animals, but the length of an incubation has a marked effect on enzyme temperature optima, and enzymes may be degraded during prolonged incubations at supposedly optimum temperatures measured during shorter incubations (Chesters and Bull, 1963; Lindley et al., 1976; Jacober et al., 1980). Since gut retention times for these South African mussels are between two and eight hours (Bayne et al., 1984) physiological temperature optima are likely to be considerably lower than those obtained for a 5 minute incubation.

Gel filtration of enzyme proteins

Tables 19 and 20 show that there was a wide range in the molecular weights of proteins exhibiting α-amylase and laminarinase activity, and a number of isozymes of these enzymes may occur in the same animal. Isozymic forms of α-amylase (Wojtowicz, 1972; Alemany and Rosell-Perez, 1973; Newell et al., 1980b; Trainer and Tillinghurst, 1982) and laminarinase (Wojtowicz, 1972; Lindley et al, 1976; Elyakova and Zvyagintseva, 1981; Stark and Walker, 1983) have been

isolated from various molluscs. Seiderer et al. (1982) reported molecular weights of 67000 and ± 9000 for crystalline style α-amylase proteins and 67000 and < 5000 for style laminarinase proteins of C. meridionalis and P. perna. In the present study, α-amylase protein molecular weights were estimated as 88000 and 48000 in C. meridionalis styles and 88000 and 39000 in P. perna styles, while style laminarinase proteins had molecular weights of 88000 and 26000 for C. meridionalis and 88000, 57000 and 28000 for P. perna. Why these estimates of molecular weights differ is not clear. Other reported α-amylase molecular weights are 39000 for Placopecten magellicanicus (Wojtowicz, 1972), and 15700 and 12900 for Mya arenaria (Trainer and Tillinghurst, 1982). Wojtowicz (1972) estimated a molecular weight of 56000 for P. magellanicus laminarinase enzyme.

In terms of cellular energy, the production of isozymes which enable an organism to utilize a changing thermal regime efficiently is a better strategy than the increased production of a less efficient pre-existing enzyme which cannot function over the whole range of temperatures at which catalysis must be performed (Hochachka and Somero, 1973). If α-amylase and laminarinase proteins eluting at several widely differing elution volumes represent isozymes of these enzymes in the same animal (see Hoffmann, 1983), then Tables 19 and 20 show that M. galloprovincialis possesses more isozymes of α-amylase both in style and digestive gland eluates than the other three mussel species, and more style laminarinase

isozymes than C. meridionalis and A. ater. In addition the M. galloprovincialis α-amylase peak with a molecular weight of 25000 has a specific activity at least three times greater than any other mussel α-amylase (Table 19). In view of the importance of α-amylase in compensating for temperature induced increases in metabolic costs, M. galloprovincialis may therefore be at a considerable advantage in coping with a changing subtidal or intertidal thermal regime. This may be a partial explanation of the relatively recent success of this species in colonizing the intertidal area on the west coast, and it may enable the species to extend its range to the warmer waters east of Cape Point.

Saccharogenesis in relation to habitat.

From the data presented above, an estimate of total saccharogenesis by crystalline style and digestive gland eluates of the different mussel species can be made. Table 21 shows the saccharogenic activity at 25^{0}C of style and digestive gland eluates of the mussels. These data were calculated from the α-amylase and laminarinase activities shown in Figures 28 and 29. However, 15^{0}C is a more reasonable reflection of environmental sea temperatures on the south and west coasts. The data were therefore adjusted to activity at 15^{0}C using correction factors calculated from the rate-temperature curves in Figures 23 - 26. The percentages of total saccharogenesis at 15^{0}C resulting from α-amylase and laminarinase activity were calculated and, as

TABLE 21

Total maltose liberation (mg maltose.h^{-1} at 25°C) from eluates of styles and digestive glands of C. meridionalis, M. galloprovincialis, P. perna and A. ater. Data is also expressed as mg.maltose liberated per mg total protein in the eluates and this was corrected to mg maltose. mg total $protein^{-1}.h^{-1}$ at 15°C using conversion factors calculated from temperature coefficients between 15°C and 25°C of Figures 23-26. The proportion of total saccharogenesis from α-amylase activity and laminarinase activity is also shown. For comparative purposes saccharogenesis per mg total protein at 15°C by style and digestive gland homogenates used for rate-temperature curves (RT) is shown.

		mg maltose.h^{-1} at 25°C	mg maltose. mg total $protein^{-1}.h^{-1}$ at 25°C	mg maltose. mg total $protein^{-1}.h^{-1}$ at 15°C	% of Total	RT mg maltose.mg $total\ protein^{-1}.h^{-1}$ at 15°C
C. meridionalis						
Style	α-amylase	46,92	4,88	2,78	42,51	
	laminarinase	55,97	5,83	3,76	57,49	
	Total			6,54	100	8,73
d. gland	α-amylase	183,36	9,17	4,68	64,82	
	laminarinase	89,22	4,46	2,54	35,18	
	Total			7,22	100	11,32
style and d. gland	α-amylase + laminarinase			13,76		20,05
M. galloprovincialis						
style	α-amylase	125,10	11,78	6,47	61,56	
	laminarinase	62,10	5,58	4,04	38,44	
	Total			10,51	100	16,11
d. gland	α-amylase	88,78	7,91	4,35	61,61	
	laminarinase	41,59	3,71	2,71	38,39	
	Total			7,06	100	7,54
style and d. gland	α-amylase + laminarinase			17,57		23,65
P. perna						
Style	α-amylase	165,17	12,41	6,54	62,05	
	laminarinase	75,56	5,68	4,09	35,95	
	Total			10,54	100	13,86
d. gland	α-amylase	29,24	3,49	2,65	63,40	
	laminarinase	17,36	2,07	1,53	36,60	
	Total			4,18	100	3,82
style and d. gland	α-amylase + laminarinase			14,72		17,68
A. ater						
style	α-amylase	35,35	3,73	1,98	42,86	
	laminarinase	36,23	3,82	2,64	57,14	
	Total			4,62	100	13,05
d. gland	α-amylase	20,76	2,82	1,97	30,12	
	laminarinase	47,35	6,44	4,57	69,88	
	Total			6,54	100	7,63
style and d. gland	α-amylase + laminarinase			11,16		20,68

an independent comparison of total saccharogenesis, the saccharogenic potential at $15^{0}C$ of the crystalline style and digestive gland homogenates is also shown in Table 21. The agreement between the two estimates of total saccharogenesis is reasonable, although there appears to be some loss in enzyme activity during elution through the column.

In crystalline style eluates saccharogenesis per unit total protein at $15^{0}C$ of M. galloprovincialis and P. perna was 10,51 mg maltose.mg protein^{-1}.h^{-1} and 10,54 mg maltose.mg protein^{-1}.h^{-1} respectively, which was considerably greater than that of C. meridionalis (6,54 mg maltose.mg protein^{-1}.h^{-1}) and A. ater (4,62 mg maltose.mg protein^{-1}.h^{-1}). M. galloprovincialis and P. perna are found in the mid intertidal zone while C. meridionalis and A. ater are restricted to the low intertidal and subtidal areas. More rapid saccharogenesis would compensate for the reduced feeding times available in the mid intertidal zone, enabling the first two species to penetrate higher into the intertidal area.

In digestive gland eluates of C. meridionalis, M. galloprovincialis and A. ater, saccharogenesis per unit total protein ranged between 6,54 and 7,22 mg maltose.mg protein^{-1}.h^{-1}, while that of P. perna was 4,18 mg maltose.mg protein^{-1}.h^{-1}. In the homogenates used for rate-temperature experiments, digestive gland saccharogenesis of the former three species was approximately twice that of

P. perna (Table 21). Thus in the west coast mussels, enzyme activity in the digestive gland plays a more important part in the digestive process than in the south coast P. perna, in which enzyme activity appears to be concentrated in the crystalline style. On the south coast the particulate load in the water column has a low organic and energy content (Griffiths, 1980b; Bayne et al., 1984) and is dominated by phytoplankton rather than detrital matter. On the west coast the particulate fraction has a higher organic content and is comprised largely of a detrital component (Stuart, 1982; Bayne et al., 1984; see Chapters I and II). Phytoplankton is likely to be more easily digested than refractory detrital material, which may need to be extensively processed in the digestive gland tubules for more complete hydrolysis. The difference in digestive gland enzyme activity between P. perna and the west coast mussels may partly explain why P. perna has not been able to colonize the west coast, where kelp beds are an integral part of the ecosystem.

The proportion of total saccharogenesis resulting from α-amylase and laminarinase activity at 15^{0}C is also of interest (Table 21). It is evident that in eluates of style proteins of C. meridionalis and A. ater, which are associated with the kelp beds, laminarinase activity accounted for 57% of total style saccharogenesis. In eluates of style proteins of M. galloprovincialis and P. perna, which are not found in the kelp beds, laminarinase activity accounted for approximately 38% of total saccharogenesis. In digestive

gland eluates of A. ater, laminarinase activity accounted for almost 70% of total saccharogenesis (Table 21). Although changing gut and style pHs will have an effect on these proportions (Fielding, unpublished data), there nevertheless appears to be some specialization for a laminarin diet in the kelp bed mussels.

Carbon budget and style turnover times

An estimate of the potential ability of crystalline style and digestive gland enzyme activity to meet the carbon requirements of the mussels may be made from the data in Table 21, provided the total protein in the styles and digestive glands of the species is known. The styles and digestive gland material from ten 60 mm (± 2 mm) shell length mussels of each species were individually removed and the protein content determined. These data are shown in Table 22. Saccharogenic activity of style and digestive gland eluates at 15^{0}C was converted to total potential maltose production using mean style and digestive gland protein contents and is shown in Table 23. Potential carbon production was then calculated on the basis of a 42% carbon content of maltose.

Carbon requirements for the animals were calculated from respiration rates. Routine respiration rates for 60 mm shell length mussels were obtained from Griffiths (1980a) for C. meridionalis, Griffiths and King (1979a) for A. ater, and Berry and Schleyer (1983) for P. perna. There are no data for

TABLE 22

Total protein content (± SD) in the crystalline styles and digestive glands of *C. meridionalis*, *M. galloprovincialis*, *P. perna* and *A. ater* of 60 mm (± 2 mm) shell length.

Mussel	Crystalline style protein (mg)	Digestive gland protein (mg)
C. meridionalis	5,48 (± 0,50)	40,00 (± 8,62)
M. galloprovincialis	6,34 (± 0,69)	52,11 (± 5,86)
P. perna	4,47 (± 1,18)	23,15 (± 4,60)
A. ater	4,09 (± 0,99)	40,87 (± 7,32)

From $y = 0,025 + 0,00097x$ ($r = 0,99$, $n = 8$) where y is the absorption at 660 nm and x is the protein concentration in $ug.ml^{-1}$, using bovine serum albumin as a standard (Lowry *et al.*, 1951).

TABLE 23

Potential carbon production by style and digestive gland eluates of C. meridionalis, M. galloprovincialis, P. perna and A. ater. Saccharogenesis at 15°C was converted to total carbon using style and digestive gland protein estimates from Table 21. Routine respiration rates of 60 mm mussels were obtained from Griffiths (1980a) for C. meridionalis, Griffiths and King (1979a) for A. ater and Berry and Schleyer (1983) for P. perna. An estimate based on values of Bayne et al. (1984) was made for M. galloprovincialis respiration. Respiration rates from the literature were adjusted to respiration rates at 15°C assuming a temperature coefficient of 2,36 (Miller, quoted by Berry and Schleyer, 1983). Respiration rates were multiplied by a factor of 2 to allow for growth (Bayne and Newell, 1983) and carbon requirements were calculated using a conversion factor of 1 ml O_2 = 0,530 mg C (Hawkins and Bayne, 1985).

	Saccharogenesis at 15°C. mg maltose.mg protein.h^{-1}	Protein content mg	* mg maltose.h^{-1}	* mg C.h^{-1} (A)	Respiration at 15°C ml O_2.h^{-1}	Carbon requirements mg C.h^{-1} (B)	a. Style turnover time to support C requirements (h) b. Potential of d.gland to support C. requirements (h) (A/B)
C. meridionalis							
Style	6,54	5,48	42,42	17,82	0,406	0,430	a 41
d. gland	7,22	40,00	188,80	121,30			b 282
			Total	139,12			
M. galloprovincialis							
Style	10,51	6,34	66,63	27,98	0,629	0,667	a 42
d. gland	7,06	52,11	367,90	154,52			b 232
			Total	182,50			
P. perna							
Style	10,54	4,47	47,11	19,79	0,168	0,178	a 111
d. gland	4,18	23,15	96,76	40,64			b 228
			Total	60,43			
A. ater							
Style	4,62	4,09	18,90	7,94	0,248	0,263	a 30
d. gland	6,54	40,87	267,29	112,26			b 427
			Total	120,20			

* Note: Carbon production.h^{-1} assumes that the entire style and digestive gland hydrolytic capacity is used at once.

respiration rates of M. galloprovincialis. Bayne et al. (1984) describe respiration rates of P. perna from Blouberg Strand. Since this species is rarely found in this area and M. galloprovincialis has been mistakenly identified as P. perna on the west coast for many years (Grant et al., 1984), there is a good chance that the data of Bayne et al., (1984) refer to M. galloprovincialis. Thus an estimate of the routine oxygen requirements of a 60 mm shell length M. galloprovincialis was made from Bayne et al.,(1984). In Table 23 respiration rates from the literature were corrected to respiration rates at 15^0C assuming a temperature coefficient of 2,36 (Miller, quoted by Berry and Schleyer, 1983; see also Bayne, 1976).

Carbon requirements have been estimated from respiration rates multiplied by a factor of two to allow for growth (Bayne and Newell, 1983) and a respiratory quotient of 1 ml O_2 = 0,530 mg C (Hawkins and Bayne, 1985).

Table 23 shows that the total hydrolytic potential of the west coast mussels C. meridionalis, M. galloprovincialis and A. ater ranges between 120,20 mgC.h^{-1} and 182,50 mgC.h^{-1} while that of the south coast P. perna is very much lower (60,43 mgC.h^{-1}). Bayne et al. (1984) have shown that the rate of food passage through the gut of mussels from the south and west coasts of South Africa is strikingly different. C. meridionalis and P. perna in False Bay have much longer gut retention times than C. meridionalis and

P. perna at Blouberg Strand. Bayne et al. (1984) postulate that in response to site specific differences in ration, mussels may increase the volume capacity of the digestive system, whilst holding clearance rates constant, and so increase gut retention time and absorption efficiency. The energetic consequences of a decline in food quality may thus be modified. Although no comparison of digestive enzymes of C. meridionalis on both sides of the Cape Peninsula was made, Table 23 indicates that differences in gut retention time of different mussel species may be a result of differences in total potential hydrolytic capabilities of the mussels' digestive systems. C. meridionalis and M. galloprovincialis from Blouberg Strand can produce 139,12 $mgC.h^{-1}$ and 182,50 $mgC.h^{-1}$ respectively by the action of their digestive enzymes. Digestive enzymes of P. perna can produce 60,43 $mgC.h^{-1}$. Thus hydrolysis of food would take considerably longer in P. perna and a longer gut retention time would be expected.

It must be noted that in Table 23, the potential carbon production is calculated on the basis of the entire style and digestive gland being used at once, and this explains why carbon production greatly exceeds carbon demand. The rate of carbon production really depends on the rate of enzyme protein production. However, this is not known and instead the style turnover times required to release sufficient maltose to meet the carbon requirements of the animals are shown, as well as the number of hours for which hydrolysis by

digestive gland enzymes could satisfy the animals' carbon requirements. Predicted style turnover times of 41 h and 111 h for C. meridionalis and P. perna respectively are in reasonable agreement with turnover times of approximately 18 h and 72 h measured by Seiderer et al. (1982). Style turnover times for M. galloprovincialis (42 h) and A. ater (30 h) are similar to that of C. meridionalis. The longer turnover time for P. perna is a result of a high style saccharogenic activity and relatively low carbon requirements (Table 23). However Table 23 suggests that by virtue of the very much greater protein content of the digestive gland compared with the crystalline style, the importance of the digestive gland in the hydrolysis of food substrates may greatly exceed that of the style. Since hydrolysis by both the style and digestive gland enzymes is an integral part of the digestive process, it is likely that in practice style turnover times are very much longer than those estimated here, in which style enzymes alone must supply the carbon requirements of the animals.

It is also evident that P. perna has both a low enzyme activity per unit total digestive gland protein (4,18 mg maltose.mg protein^{-1}.h^{-1}) and approximately half the total digestive gland protein of the other mussel species (Tables 21 and 22. Potential carbon production by digestive gland enzymes of P. perna is 40,64 mgC.h^{-1} compared with values ranging between 112,26 mgC.h^{-1} and 154,52 mgC.h^{-1} for the other species (Table 23). Thus in P. perna the style is

required to play a relatively more important part in saccharogenesis than in C. meridionalis, M. galloprovincialis and A. ater, and turnover time may well be considerably shorter than in the three west coast mussels.

The data in Table 23 may provide some explanation for the recent success of M. galloprovincialis in colonizing the west coast. Potential carbon production by style and digestive gland enzymes of this species is 182,50 $mgC.h^{-1}$ while for C. meridionalis and A. ater values are 139,12 $mgC.h^{-1}$ and 120,20 $mgC.h^{-1}$ respectively. Carbon requirements estimated for M. galloprovincialis in Table 23 are speculative. If they are in fact similar to those of C. meridionalis, M. galloprovincialis would be able to hydrolyse sufficent food for basic metabolic requirements considerably faster than C. meridionalis, which would give it a competitive advantage. Provided aerial respiration rates of the two species were similar, M. galloprovincialis would be able to colonize rocks higher up the intertidal zone, where feeding times are limited. The slow clearance rates of A. ater (Stuart, 1982; Bayne et al., 1984) are probably responsible for the lack of success of this species intertidally, since for similar sized animals potential carbon production (120,20 mg $C.h^{-1}$) is not very different from that of C. meridionalis (139,12 $mgC.h^{-1}$).

CONCLUSIONS

P. perna is associated with a food source consisting mainly of phytoplankton on the south and east coasts, and has a high crystalline style saccharogenic potential (10,54 mg maltose.mg total protein^{-1}.h^{-1}) compared with the west coast mussels C. meridionalis and A. ater, which have style hydrolytic potentials of 6,54 mg maltose.mg total protein^{-1}.h^{-1} and 4,62 mg maltose.mg total protein^{-1}.h^{-1} respectively (Table 21). Saccharogenic potential of M. galloprovincialis style enzymes (10,51 mg maltose.mg total protein^{-1}.h^{-1}) is very similar to that of P. perna. If a high style enzyme activity is necessary for the successful utilization of a principally phytoplankton diet, then M. galloprovincialis is well suited to colonize the south and east coasts. The high potential total carbon production by digestive gland enzymes, a high style enzyme activity per unit total protein, a considerable number of possible α-amylase isozymes in both the crystalline style and digestive gland proteins, and high temperature coefficients for style and digestive gland α-amylases, may all contribute to make M. galloprovincialis a very successful competitor both intertidally and subtidally on the South African coastline. P. perna may be prevented from colonizing the west coast by low digestive gland carbohydrase activities which limit its ability to utilize a largely detrital food source, while C. meridionalis and A. ater have carbohydrase activities well suited to hydrolyse the laminarin storage

products of both the macrophytes and diatoms which occur in the kelp beds (Chapter II). However, in the coastal environment, mussel digestive polysaccharases are not usually required to hydrolyse a pure glycogen or laminarin substrate, but are required to act on a generally refractory particulate food source. The action of these enzymes on natural detrital and algal food sources is examined in the next section.

SECTION III

DIGESTION AND UTILISATION OF AVAILABLE RESOURCES BY SOUTH AFRICAN MUSSELS

Data presented in the previous two sections lead to the conclusion that particulate resources are dominated by detrital material and that style and digestive gland enzymes can release a significant fraction of the mussels' energy requirements by hydrolysis of commercial substrates. It would seem also that the digestive gland may be more important than the crystalline style as a source of hydrolytic enzymes. Nevertheless two major problems emerge. Firstly, the analytical methods employed significantly influence the estimate of enzyme hydrolytic activity. Secondly, calculations of energy released by enzyme activity are complicated by uncertain estimates of rates of style and digestive gland enzyme release.

However, tests on the digestibility of purified substrates are uncomplicated by biochemical components of detrital material such as polyphenols, tannins, silica-cellulose complexes (diatoms) and attached bacteria, which may interfere with or be resistant to the digestive enzymes of mussels. Detrital material is known to be dominated also by celluloses, which many organisms are largely unable to digest. Organisms have generally overcome this problem either by using bacteria to break down the cellulose for them in their digestive tracts (termites, ruminants), or the organism has consumed the bacteria themselves as a significant energy resource, as Newell (1965) demonstrated in a classical study on the nutrition of the prosobranch mollusc Hydrobia ulvae. The following section examines the ability of mussel

digestive enzymes to release sufficient energy from the hydrolysis of natural particulate suspensions, and investigates also the contentious role of free, attached and gut bacteria in mussel nutrition. An attempt is also made to resolve one of the original hypotheses. Can the distribution of mussel species be ascribed to resource partitioning on the basis of differing abilities to utilise the food resource effectively?

CHAPTER V

HYDROLYSIS OF ALGAL AND DETRITAL FOOD SUBSTRATES BY MUSSEL DIGESTIVE ENZYMES

INTRODUCTION

Digestive enzymes in bivalves have been widely reported, and in South African mussels they have been investigated in some detail (Seiderer et al., 1982; see Chapter IV). Data in Chapter IV have shown that hydrolysis of glycogen and laminarin substrates by digestive carbohydrases of C. meridionalis, M. galloprovincialis, P. perna and A. ater can supply the carbon requirements of these animals. However, food supplies in the natural environment are not of a pure commercially prepared nature. In living phytoplankton, carbohydrate storage products are locked inside cells which are often enclosed in a cellulose cell wall. Much of the < 200 um particulate fraction in the water column of the South African west coast inshore area is of a detrital, refractory nature (Stuart, 1982; see Chapters I and II), and consists mainly of structural carbohydrates.

The ability of mussels to utilize this particulate food source has been shown by the work of Griffiths (1980b), Stuart et al. (1982) and Berry and Schleyer (1983). In these studies, C. meridionalis, P. perna and A. ater have been shown to absorb 40-61% of the organic component of naturally occurring particulates. Before absorption can occur the complex polysaccharides constituting such material must be broken down to component monomers by the activity of digestive enzymes.

Lucas and Newell (1984) showed that the bivalves Crassostrea virginica and Geukensia demissa possess crystalline style enzymes, capable of liberating reducing sugars from naturally occurring detrital and salt marsh grass substrates, and suggested that approximately 40% of the carbon absorption requirements of C. virginica could be met by the utilisation of salt marsh grass detritus. However, the molecular weight of the reducing sugar used as a standard has a marked effect on the calibration curve and this affects the quantitative estimate of sugar release necessary for the calculation of energy budgets. The percentage of carbon requirements of C. virginica met by style enzyme hydrolysis of salt marsh grass particles may be closer to 13% (see Chapter III). However, digestive gland enzymes were not examined by Lucas and Newell (1984). The importance of the digestive gland in the hydrolysis of food substrates may greatly exceed that of the style (see Chapter IV). It is therefore of interest to see what in vitro reducing sugar release is possible by digestive enzymes of the mussels that colonise the South African coastline, given a substrate with which the digestive systems would have to contend in the natural environment. Reducing sugar release may then be compared with the carbon requirements of the animals. A comparison may also be made between style turnover times necessary to meet the carbon requirements of the animals given a naturally occurring substrate, and turnover times estimated from the release of reducing sugars from pure glycogen and laminarin.

Hydrolysis of the constituents of living phytoplankton cells may be easier than hydrolysis of the mainly structural carbohydrates in a detrital substrate. Thus it is also of interest to determine whether mussel digestive enzymes can lyse algal cells, since there is no information on the effect of digestive enzymes on living phytoplankton.

The following work was carried out to determine the rates of hydrolysis of natural particulate material by mussel digestive enzymes and to examine the effect of these enzymes on different algal cells. The extent to which an analysis of hydrolytic rates of digestive enzymes can contribute to an understanding of the feeding ecology of these animals can then be assessed.

MATERIALS AND METHODS

Hydrolysis of natural detrital material

Enzyme preparation

Initially the release of reducing sugars from a variety of substrates by purified enzymes was investigated and compared with reducing sugar release by mussel crystalline style enzymes. Enzymes were -amylase from Bacillus subtilus (Sigma, bacterial crude type 3), laminarinase from

Penicillium species (Sigma, No. L-9259) and a composite cellulase from Aspergillus niger (Sigma, practical grade 2). Enzymes were prepared in 20 mM phosphate buffer pH 6,9, containing 150 mM NaCl, at concentrations of 0,125 $mg.ml^{-1}$ for α-amylase and laminarinase and 0,5 $mg.ml^{-1}$ for cellulase. C. meridionalis, M. galloprovincialis and A. ater were collected from the intertidal rocks at Blouberg Strand on the west coast of South Africa, and P. perna were collected intertidally at Bailey's Cottage in False Bay. Solutions of mussel crystalline style and digestive gland enzymes were prepared in 20 mM phosphate buffer pH 6,9 containing 150 mM NaCl, as described in Chapter IV. Protein concentrations of enzyme solutions were determined by the method of Lowry et al. (1951) from the equation $y = 0,035 + 0,0011x$ ($r = 0,99$, $n = 7$) where y is the optical density at 660 nm and x is the protein concentration in $\mu g.ml^{-1}$, using bovine serum albumin as a standard.

Substrate Preparation

Commercial substrates were prepared in 20 mM phosphate buffer pH 6,9 containing 150 mM NaCl at the following concentrations: oyster glycogen (BDH NO. 38042) 1% w/v, laminarin (Sigma No. L9634) 0,4% w/v, carboxymethyl cellulose (CMC, BDH) 1% w/v, and alginic acid (Sigma, No. A7128) made up as sodium alginate 0,4% w/v. Naturally occurring substrates were made from kelp fronds and a phytoplankton/ detrital mixture centrifuged from sea water. Kelp frond tips were sliced from macrophytes at Oudekraal on the west coast

of the Cape Peninsula, and were lyophilised and then powdered with a mortar and pestle to pass through a 90 µm sieve. A phytoplankton/detrital concentrate, representing food very similar to that naturally available to filter feeders, was obtained by backwashing a large aquarium pump filter (0,45 µm). Water pumped through the filter was taken directly from the sea from a site approximately 5 km north of Oudekraal. The concentrate was centrifuged at 9000 x g for 10 minutes, resuspended in ammonium formate isotonic with sea water and centrifuged a further three times to remove NaCl. Thereafter, the detrital material was lyophilized and powdered to pass through a 90 µm sieve. Kelp and natural detrital substrates were made up in phosphate buffer at a concentration of 2% w/v.

Initial experiments with kelp and natural detrital substrates yielded high blank values, reaching 1,64% and 0,32% maltose equivalents by dry weight for kelp and natural detritus, respectively. Dialysis for 20 hours against 2000 volumes of chilled stirred phosphate buffer with one change, reduced kelp blank values by 48% and natural particulate detrital blank values by 27%. Kelp frond substrates were therefore dialysed before use in enzyme assays. Refluxing the natural particulate detritus with NaOH reduced blank values by 38% to 0,27% w/v maltose. However, refluxing this material as suggested by Lucas and Newell (1984), changes the nature of the substrate, reducing it to mainly cellulose and hemicellulose residues. Some experiments performed using

natural detritus were repeated using refluxed material. Particulate detrital material was refluxed at 100°C in 100 ml 50 mM NaOH for four hours, and the residue was resuspended, centrifuged and dried following the procedure of Lucas and Newell (1984). Thereafter it was powdered to pass through a 90 µm sieve and a 2% solution made up in phosphate buffer.

Reducing Sugar Assays

Assays for reducing sugars released by enzyme action on substrates were conducted according to the method of Nelson (1944) modified by Somogyi (1952), as described in Chapter III. However, assays were conducted at 12,5°C since this is close to the mean summer temperature of the west coast inshore waters (see Chapter I), and the incubation period was 10 minutes. Reducing sugar release was calculated from the equation $y = 0,02 + 6,24x$ ($r = 0,99$, $n = 5$) where y is the absorbance at 660 nm and x is the reducing sugar concentration in $mg.ml^{-1}$, using maltose as a standard. Enzyme and substrate blanks were subtracted from experimental values and all data given in the results are the mean of triplicate readings.

The rate of release of reducing sugars from kelp and natural detrital substrates by both microbial and crystalline style enzymes was very low, so a series of rate-time and rate-temperature enzyme assays, using natural detrital substrate, were undertaken. For these experiments, bacterial α-amylase and cellulase concentrations were increased to 1 $mg.ml^{-1}$,

while laminarinase solutions were maintained at 0,125 $mg.ml^{-1}$ because of a lack of sufficient enzyme. Reducing sugar assays were also carried out with cellulase enzyme solutions of 2 $mg.ml^{-1}$ and 10 $mg.ml^{-1}$. Protein concentrations of mussel style enzyme solutions were increased to between 1,062 and 3,673 mg $protein.ml^{-1}$, while concentrations of digestive gland enzyme solutions ranged between 0,117 mg $protein.ml^{-1}$ and 0,687 mg $protein.ml^{-1}$. The release of reducing sugars from natural particulate detritus by microbial enzymes, mussel crystalline style and digestive gland enzymes were measured as described above, over periods of between 2 minutes and 120 minutes, and the release of reducing sugars from the same substrates by bacterial enzymes and style enzymes was measured between temperatures of 5°C and 40°C. Rate-time assays were conducted at 12,5°C. Rate-temperature assays had incubation periods of 10 minutes.

Extended incubation times and elevated temperatures did little to increase reducing sugar release from a natural detrital substrate. To test the possibility that other enzymes in the digestive system may play a role in the hydrolysis of this substrate, the entire digestive system comprising oesophagus, stomach, crystalline style and sac, digestive gland and lower intestine, was dissected from five M. galloprovincialis freshly collected from Blouberg Strand. The digestive systems were homogenised together in 65 ml phosphate buffer, centrifuged at 30000 x g for 60 minutes and dialysed against 3000 volumes of chilled stirred phosphate

buffer for 20 hours. The reducing sugar release from natural particulate detrital material (2% w/v) by the resultant enzyme solution was measured at 25°C for incubation periods of between 2 minutes and 120 minutes.

The release of reducing sugars over time by style enzyme solutions of C. meridionalis, M. galloprovincialis, P. perna and A. ater prepared as previously described, from refluxed natural particulate detrital material, was also measured by the Nelson-Somogyi method. Incubation temperature was 12,5°C.

Action of enzymes on phytoplankton

Two diatom species, Chaetoceros gracilis and Thallassiosira weissflogii, and two flagellates, Tetraselmis suecica and Dunaliella primolecta, were selected for examination of the effect of digestive enzymes on living algal cells. Chaetoceros and Thallassiosira species occur frequently in the west coast phytoplankton assemblages (Barlow, 1982; Probyn, 1985) while Tetraselmis and Dunaliella species possess thin cell walls and have often been used to measure bivalve absorption efficiencies (see Winter, 1978; Griffiths and King, 1979a; Griffiths, 1980a). A preliminary microscopic examination of the gut contents of C. meridionalis and M. galloprovincialis, which were first starved in filtered sea water and then fed these algae, showed that in the mussel gut, a rapid leaching of chlorophyll from the cell and distortion of cell shape

occurred. Thus changes in the numbers of undamaged cells in an algal-enzyme incubation were assessed using a standard light microscope and a magnification of 400X

Enzyme preparation

C. meridionalis and M. galloprovincialis style and digestive gland extracts with protein concentrations of 5 $mg.ml^{-1}$ were prepared in 20 mM phosphate buffer pH 6,9, containing 150 mM NaCl as described in Chapter IV. Similar digestive enzyme extracts were prepared in 0,2 µm filtered sea water pH 8,0, and 0,2 µm filtered sea water in which the pH was lowered to 6,5 with 0,01 M HCl. Cellulase extracts (Aspergillus niger, 1 $mg.ml^{-1}$) were prepared in phosphate buffer, sea water pH 8,0 and sea water pH 6,6.

Algal incubations

Aliquots of actively growing unialgal cultures of Chaetoceros, Tetraselmis, Thallassiosira and Dunaliella were centrifuged (3000 x g for 10 minutes) and pellets were resuspended in phosphate buffer, 0,2 µm filtered sea water pH 8,0 and 0,2 µm filtered sea water pH 6,5. Exactly 500 µl of algal suspension was mixed with 500 ul of the appropriate enzyme extract for a final cell concentration of approximately 5 x 10^6 $cells.ml^{-1}$ for Chaetoceros, Tetraselmis and Dunaliella, and 1,75 x 10^6 $cells.ml^{-1}$ for Thallassiosira. Incubations were shaken for 8 hours at 20^{o} C. Control

incubations consisted of algae resuspended in 1 ml sea water pH 8,0.

Counting procedure

Immediately after mixing of the algal suspension and enzyme extract, and then again after 8 hours, the numbers of normal cells in an incubation were quantitatively assessed using a haemocytometer cell with improved Neubauer rulings. A minimum number of 400 cells were counted (in at least 18 fields) for Chaetoceros, Tetraselmis and Dunaliella. For Thallassiosira, a minimum of 50 fields were counted because of the lower cell concentrations. This was shown to reduce the standard error of the mean to less than 5 % (Figure 30). Numbers of normal cells at the start and end of the incubation were compared using a Students t-test. X^2 tests for randomness of cell distribution in the haemocytometer cell were conducted on five data sets for each algal species, using the method of Lund et al. (1958).

RESULTS

Hydrolysis of natural detrital material

The release of reducing sugars from a series of commercial and natural substrates by both microbial enzymes and mussel style enzymes is shown in Table 24. Preparations of

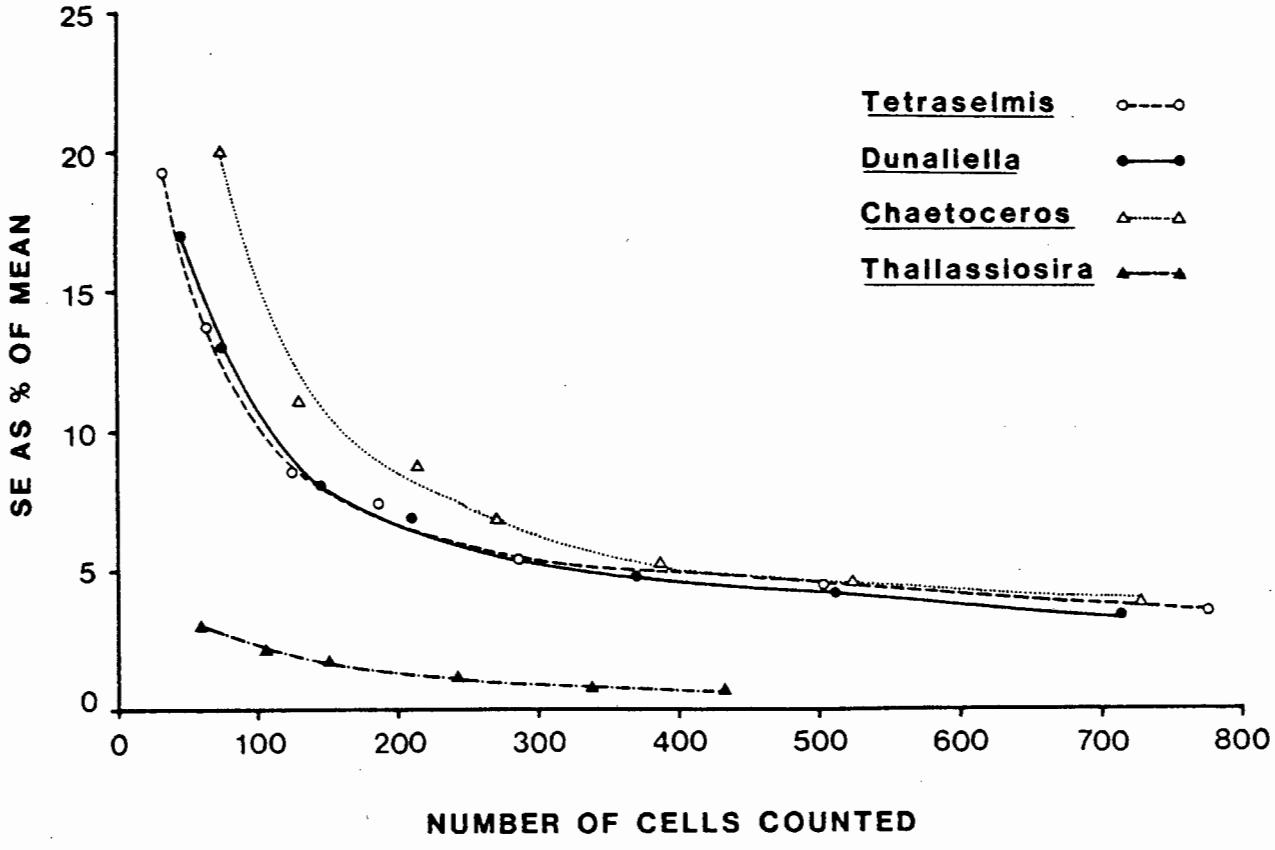


Figure 30. The relationship between the standard error as a percentage of the mean, and the number of algal cells counted using a haemocytometer cell.

TABLE 24

Release of reducing sugars from different substrates by α-amylase (0,125 mg.ml^{-1}), laminarinase (0,125 mg.ml^{-1}), cellulase (0,5 mg.ml^{-1}), and style extract of four mussel species. Substrate concentrations were 1% w/v for glycogen and CMC, 0,4% w/v for laminarin and sodium alginate and 2% w/v for kelp and natural detritus. Values represent reducing sugars released after 10 min incubation at 12,5°C and are expressed as mg maltose.mg enzyme^{-1}.h^{-1} for α-amylase, laminarinase and cellulase, and mg maltose.mg style protein^{-1}.h^{-1} for mussel style extracts.

Enzyme	Substrate					
	Glycogen	Laminarin	CMC	Alginate	Natural Detritus	Kelp
α-amylase	9,285	0,108	0,161	0,081	0,012	0,054
Laminarinase	0,088	3,233	0,169	0,088	0,014	0,074
Cellulase	0,673	0,090	0,049	0,023	0,007	0,055
C. meridionalis style	3,276	0,846	0,014	0,004	0,009	0,014
M. galloprovincialis style	3,654	0,884	0,043	0,021	0,014	0,007
P. perna style	4,902	1,434	0,028	0,001	0,003	0,013
A. ater style	1,674	1,044	0,032	0,013	0,004	0,013

microbial enzymes hydrolysed all four commercial substrates to some degree, but rates of hydrolysis of glycogen and laminarin were considerably higher that those of CMC and sodium alginate, which are similar to structural polysaccarides. Mussel style enzymes also hydrolysed glycogen and laminarin very much more rapidly than CMC and sodium alginate. Style enzyme hydrolysis of these latter two substrates ranged between 0,001 mg maltose.mg protein^{-1}.h^{-1} and 0,043 mg maltose.mg protein^{-1}.h^{-1}, while rates of hydrolysis of glycogen and laminarin reached 4,902 mg maltose.mg protein^{-1}.h^{-1} and 1,434 mg maltose. mg protein^{-1}.h^{-1}, respectively. It is of interest that microbial enzymes were not entirely substrate specific, and reducing sugars were released by α-amylase from both laminarin and CMC, by laminarinase from glycogen and CMC and by cellulase from glycogen and laminarin. Thus in a homogenate of mussel crystalline styles, in which a number of enzymes occur simultaneously, up to 8% of the reducing sugars considered to result from α-amylase activity on glycogen, may be a result of reducing sugar release by laminarinase and cellulase enzymes from glycogen. Six percent of total reducing sugar release from laminarin may be a result of α-amylase and cellulase hydrolysis of the substrate. This factor assumes some importance when reducing sugar release from CMC is considered, since laminarinase and α-amylase each liberate approximately three times more reducing sugar from CMC than cellulase, and together account for 87% of the total reducing sugar release by the three enzymes.

Rates of reducing sugar release by all enzyme preparations from natural substrates were very low. Microbial enzymes liberated between 0,007 mg maltose.mg enzyme^{-1}.h^{-1} and 0,014 mg maltose.mg enzyme^{-1}.h^{-1} from a natural detrital substrate, and between 0,054 mg maltose.mg enzyme^{-1}.h^{-1} and 0,074 mg maltose.mg enzyme^{-1}.h^{-1} from kelp particles (Table 24). Laminarinase had the highest rate of reducing sugar release from kelp (0,074 mg maltose.mg enzyme^{-1}.h^{-1}), but rates of hydrolysis of natural detritus were very similar for α-amylase, cellulase and laminarinase. Mussel style enzymes had rates of reducing sugar release from both detrital and kelp particles that did not exceed 0,014 mg maltose.mg protein^{-1}.h^{-1}, and rates of hydrolysis of both kelp and detritus were very similar. Thus, at environmental temperatures, natural detrital and kelp particulate material is not readily hydrolysed by mussel style enzymes.

Gut retention time of the mussels varies between 1,40 hours and 8,34 hours and may depend on ration (Bayne et al., 1984) and the potential hydrolytic capabilities of the digestive system (Chapter IV). Extended incubation times might be expected to provide greater reducing sugar release. The results of microbial enzyme incubations with natural detrital substrates for up to 120 minutes are shown in Table 25. In spite of an increase in microbial enzyme concentrations to 1 mg enzyme.ml^{-1} for α-amylase and cellulase, there was no

TABLE 25

Release of reducing sugars (mg maltose.mg enzyme^{-1}) as a function of incubation time (min) at 12,5°C by 1 mg.ml^{-1} α-amylase and cellulase, and 0,125 mg.ml^{-1} laminarinase from natural detrital material (2% w/v). Reducing sugar release by cellulase enzyme at concentrations of 2 mg.ml^{-1} and 10 mg.ml^{-1} is also shown.

Enzyme	Time (mins)									
	2	5	8	12	15	20	30	60	90	120
α-amylase	0,019	0,013	0,023	0,011	0,020	0,011	0,021	0,021	0,014	0,024
Laminarinase	0,000	0,029	0,038	0,028	0,038	0,031	-	-	-	-
Cellulase	0,006	0,006	0,002	0,001	0,001	0,001	-	-	-	-
Cellulase 2 mg.ml^{-1}	0,003	-	-	-	-	-	0,003	0,002	0,001	-
Cellulase 10 mg.ml^{-1}	0,001	-	-	-	-	-	-	0,001	0,001	0,001

readily identifiable increase in reducing sugar release with time. Microbial cellulase concentrations of 2 mg enzyme.ml^{-1} and 10 mg enzyme.ml^{-1} released a maximum of 0,003 mg maltose.mg $enzyme^{-1}.h^{-1}$ in incubation periods of between 30 minutes and 120 minutes.

Rates of reducing sugar release by mussel crystalline style and digestive gland enzymes from a natural detrital substrate are shown in Table 26. Style enzymes of C. meridionalis released 0,022 mg maltose.mg $protein^{-1}$ in 120 minutes, while those of M. galloprovincialis, P. perna and A. ater release approximately 0,004 mg maltose.mg $protein^{-1}$ over the same time period. Release of reducing sugars by digestive gland enzymes was greater and ranged between 0,024 mg maltose.mg $protein^{-1}$ and 0,064 mg maltose. mg $protein^{-1}$ after 120 minutes. After 120 minutes, digestive gland enzymes of A. ater, which is the dominant bivalve in the kelp bed systems off the west coast of South Africa, had released approximately twice as much reducing sugar (0,064 mg maltose.mg $protein^{-1}$) as the other three mussel species, whose reducing sugar release by digestive gland enzymes was very similar (between 0,024 mg maltose.mg $protein^{-1}$ and 0,032 mg maltose. mg $protein^{-1}$) for the same time period. Experimental increases in substrate concentrations did not increase rates of reducing sugar release in the incubations. Hydrolysis of a natural detrital substrate by a whole digestive system preparation of M. galloprovincialis was slow and after 120

TABLE 26

Release of reducing sugars (mg maltose.mg protein^{-1}) from natural detrital material (2% w/v) by style and digestive gland extracts of four mussel species, as a function of incubation time (min) at 12,5°C. Reducing sugar release by an extract of whole digestive system of M. galloprovincialis at a temperature of 25°C is also shown.

Enzyme	T°C	Time								
		2	5	8	12	15	20	60	90	120
C. meridionalis	12,5									
style		0,004	0,012	0,008	0,006	0,010	0,010	0,002	0,012	0,022
digestive gland		0,018	-	0,004	-	0,028	-	0,026	0,024	0,024
M. galloprovincialis	12,5									
style		0,000	0,002	0,002	0,002	0,002	0,002	0,004	0,002	0,004
digestive gland		0,000	0,002	0,004	0,008	0,024	0,038	0,026	0,024	0,032
P. perna	12,5									
style		0,002	0,002	0,004	0,006	0,004	0,002	0,004	0,004	0,004
digestive gland		0,002	0,011	0,006	0,010	0,016	0,012	0,020	0,024	0,024
A. ater	12,5									
style		0,002	0,002	0,006	0,006	0,006	0,008	0,006	0,006	0,004
digestive gland		0,009	0,018	0,014	0,017	0,029	0,014	0,028	0,045	0,064
M. galloprovincialis	25									
whole digestive system		0,004	0,006	0,006	0,004	0,006	0,006	0,002	-	0,002

minutes did not exceed 0,006 mg maltose.mg protein^{-1} (Table 26).

Rates of reducing sugar release by both microbial enzymes and crystalline style enzymes generally increased slightly with temperature (Table 27). A maximum rate of 0,033 mg maltose.mg enzyme^{-1}.h^{-1} was reached at 40°C by α-amylase, while for laminarinase and cellulase, values increased from approximately 0,010 mg maltose.mg enzyme^{-1}.h^{-1} at 5°C to approximately 0,020 mg maltose.mg enzyme^{-1}.h^{-1} at 40°C. Mussel style enzymes showed a similar pattern, with hydrolysis rates of 0,004 - 0,006 mg maltose.mg protein^{-1}.h^{-1} at 5°C, and 0,014-0,020 mg maltose.mg protein^{-1}.h^{-1} at 40°C. In the west coast environmental temperature range of approximately 10°C to 15°C (see Chapter I), rates of reducing sugar release by crystalline style enzymes ranged between 0,008 mg maltose.mg protein^{-1}.h^{-1} and 0,014 mg maltose. mg protein^{-1}.h^{-1}.

Rates of reducing sugar release by mussel style enzymes from refluxed natural detrital material are shown in Table 28. Rates were very similar to those shown in Table 26 for material that had not been refluxed, and did not exceed 0,006 mg maltose.mg protein^{-1} after a 20 minute incubation.

Action of enzymes on phytoplankton

X^2 tests showed that algal cells were randomly distributed throughout the haemocytometer cell ($p < 0,05$). Significant

TABLE 27

Release of reducing sugars from natural detrital mateial (2% w/v) as a function of temperature by α-amylase (1 $mg.ml^{-1}$), laminarinase (0,125 $mg.ml^{-1}$) and style extract of four mussel species. Values represent reducing sugars released after 10 min inacubation and are expressed as mg.maltose.mg $enzyme^{-1}.h^{-1}$ for α-amylase, laminarinase and cellulase, and mg.malfose.mg $protein^{-1}.h^{-1}$ for style extracts

Enzyme	Temperature °C							
	5	10	15	20	25	30	35	40
α-amylase	0,021	0,014	0,024	0,023	0,021	0,032	0,026	0,033
Laminarinase	0,010	0,013	0,013	0,017	0,014	0,016	0,017	0,018
Cellulase	0,012	0,016	0,011	0,017	0,024	0,012	0,025	0,023
C. meridionalis style	0,005	0,012	0,012	0,012	0,012	0,012	0,014	0,016
M. galloprovincialis style	0,004	0,008	0,014	0,012	0,012	0,014	0,014	0,020
P. perna style	0,006	0,014	0,014	0,016	0,015	0,020	0,016	0,014
A. ater style	0,006	0,012	0,010	0,022	0,030	0,016	0,022	0,016

TABLE 28

Release of reducing sugars (mg.maltose.mg protein^{-1}) as a function of incubation time (min) at 12,5°C from refluxed (0,05 M NaOH for four hours) natural detrital material (2% w/v) by style extracts of four mussel species.

Enzyme extract	Time (mins)					
	2	5	8	12	15	20
C. meridionalis style	0,004	0,006	0,004	0,006	0,004	0,004
M. galloprovincialis style	0,002	0,004	0,004	0,006	0,006	0,006
P. perna style	0,002	0,006	0,004	0,004	0,006	0,006
A. ater style	0,004	0,004	0,004	0,004	0,004	0,004

differences in the numbers of normal cells after incubations with various enzyme extracts are shown in Table 29. In phosphate buffer extracts, style and digestive gland enzymes of both C. meridionalis and M. galloprovincialis alter the appearance of Chaetoceros, Tetraselmis and Thallassiosira. Pigments were leached from the cell (see Figure 31) but there was no evident cell breakdown. Cellulase in phosphate buffer solutions also leached pigments from Chaetoceros and Thallassiosira. Dunaliella was affected only by M. galloprovincialis style enzyme solutions (Table 29), cells being immobilized and changing their shape and colour (see Figure 31 C and F). In all other experimental solutions, it was necessary to immobilize Dunaliella cells for counting purposes by adding 4 µl of 25 % gluteraldehyde to 250 µl of suspension.

Conditions in the bivalve gut are likely to be similar to extracts of enzymes in sea water rather than phosphate buffer, with stomach pHs ranging from 5,5 to 7,2 (Langton and Gabbott, 1974; Mathers, 1974; Langton, 1977). Compared with digestive enzymes in phosphate buffer, Table 29 shows that the effect of style and digestive gland extracts in sea water of pHs 8,0 and 6,5 was considerably reduced. Style extracts in sea water had no effect on any of the algae, and Tetraselmis and Dunaliella both required immobilization with gluteraldehyde before counting. Digestive gland extracts of both mussel species leached pigments from Thallassiosira, and M. galloprovincialis digestive gland extracts similarly

TABLE 29

Significant differences (T-test $p < 0,05$) in normal cell counts of four algal species after 8 hours incubation at 20°C in various enzyme solutions. Significant differences are marked with a +.

Treatment	Chaetoceros gracilus	Tetraselmis suecica	Thallassiosira weisfloggi	Dunaliella primolecta
C. meridionalis styles in buffer pH 7,0	+	+	+	-
C. meridionalis styles in sea water pH 6,5	-	-	-	-
C. meridionalis styles in sea water pH 8,0	-	-	-	-
Control	-	-	-	-
M. galloprovincialis styles in buffer pH 7,0	+	+	+	+
M. galloprovincialis styles in sea water pH 6,5	-	-	-	-
M. galloprovincialis styles in sea water pH 8,0	-	-	-	-
Control	-	-	-	-
C. meridionalis d.gland in buffer pH 7,0	+	+	+	-
C. meridionalis d.gland in sea water pH 6,5	-	-	+	-
C. meridionalis d.gland in sea water pH 8,0	-	-	+	-
Control	-	-	-	-
M. galloprovincialis d.gland in buffer pH 7,0	+	+	+	-
M. galloprovincialis d.gland in sea water pH 6,5	+	-	+	-
M. galloprovincialis d.gland in sea water pH 8,0	+	-	+	-
Control	-	-	-	-
Cellulase 1 $mg.ml^{-1}$ in P. buffer pH 7,0	+	-	+	-
Cellulase 1 $mg.ml^{-1}$ in sea water pH 6,5	-	-	-	-
Cellulase 1 $mg.ml^{-1}$ in sea water pH 8,0	-	-	-	-
Control	-	-	-	-
Phosphate buffer ph 7,0	-	-	-	-
Sea water pH 5,5	-	-	-	-
Sea water pH 6,5	-	-	-	-
Sea water pH 8,0	-	-	-	-

FIGURE 31

A Normal Thallassiosira cells.

B Normal Tetraselmis cells

C Normal Dunaliella cells

D Thallassiosira cells showing typical leaching of pigments from the cell after 8 h incubation in C. meridionalis and M. galloprovincialis style and digestive gland enzymes.

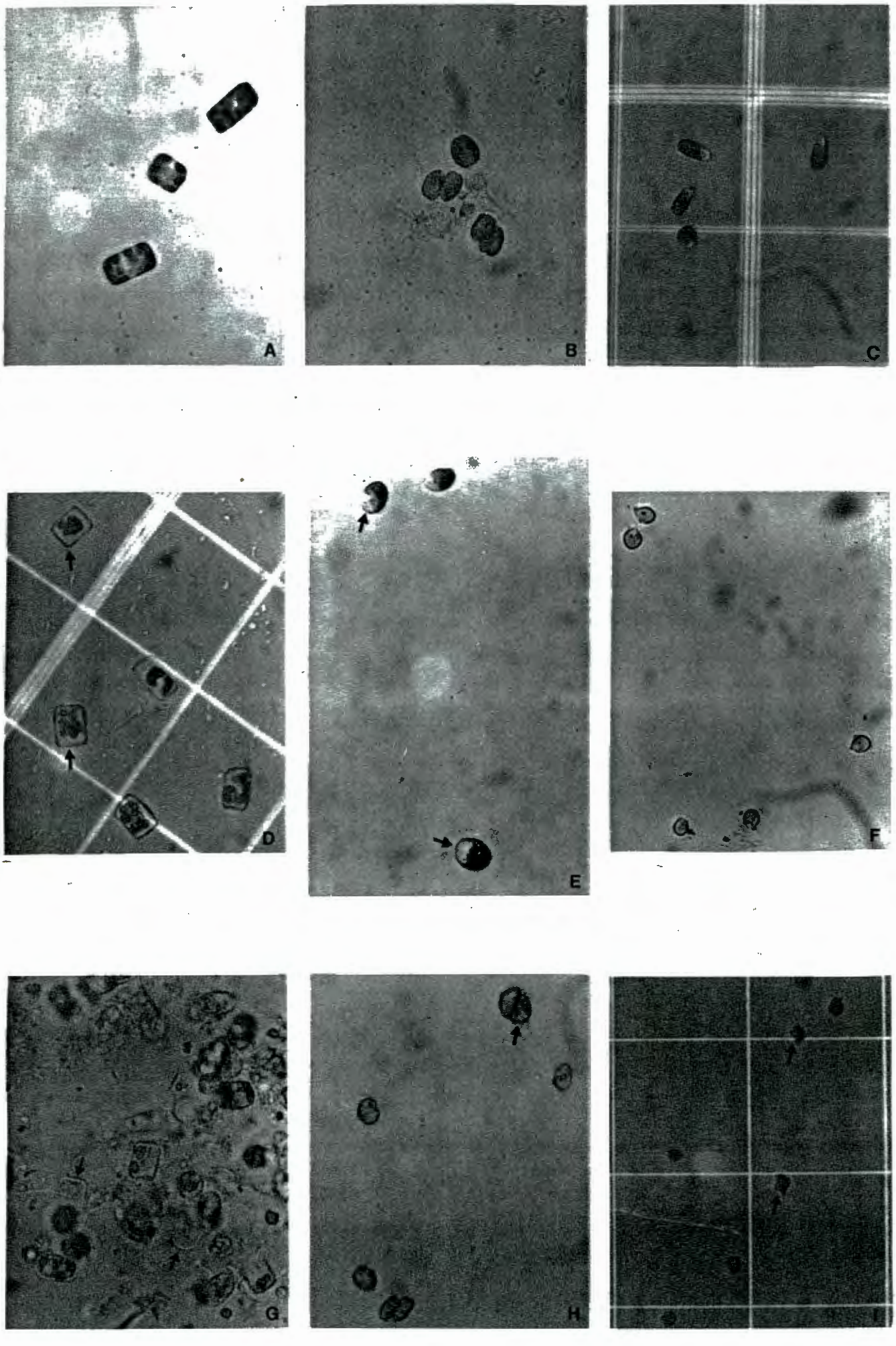
E Tetraselmis cells showing typical leaching of pigments from the cell after 8 h incubation in C. meridionalis and M. galloprovincialis style and digestive gland enzymes.

F Dunaliella cells after 8 h incubation in M. galloprovincialis style extract. Cells lose colour and changes in cell shape have occurred.

G Thallassiosira cells 30 min after ingestion by M. galloprovincialis. Empty broken cells are clearly visible.

H Tetraselmis cells 30 min after ingestion by M. galloprovincialis. Cell contents have been leached out and cells are starting to break down.

I Dunaliella cells 30 min after ingestion by M. galloprovincialis. Cells are being broken down.


A
B
C
D
E
F
G
H
I

affected Chaetoceros cells. Diatom species therefore appear to be more susceptible to the effects of enzyme activity than the flagellates. Cellulase in sea water had no effect on any of the algal cells (Table 29). Phosphate buffer alone caused slight leaching of pigments from Thallassiosira and Chaetoceros, while sea water with pHs ranging from 5,5 to 8,0 did not affect any of the cells.

Starved M. galloprovincialis were allowed to feed on the different cell types for 15 minutes and were then transfered to filtered sea water. Thirty minutes after the commencement of feeding, algal cells were photographed from gut smears and are shown in Figure 31 G, H and I. In in vitro incubations pigments were leached from cells and Chaetoceros cells became difficult to count, but even after 8 hours no cell breakdown was ever observed (Figure 31 D, E and F). However, after a maximum of 30 minutes in the mussel gut, cells were starting to break down and sometimes had lost their contents (Figure 31 G, H and I).

DISCUSSION

Hydrolysis of natural detrital material

The results presented in Table 24 show that although mussel crystalline style enzymes can readily release reducing sugars from purified commercial substrates at environmental

temperatures, hydrolysis of naturally occurring kelp and detrital particulate material was very slow. Microbial α-amylase, laminarinase and cellulase enzymes also hydrolysed these latter substrates slowly. Both style and microbial enzymes were able to hydrolyse kelp particulate matter more rapidly than detrital particles collected from the water column. Hydrolysis of natural particulate material by all enzyme preparations was not significantly increased by extended incubation periods and only very slightly by elevated temperatures (Tables 25, 26 and 27). Style enzyme hydrolysis rates of refluxed material consisting mainly of structural carbohydrates (Table 28), were very similar to rates of hydrolyis of non-refluxed material (Table 26).

Provided that the total protein in the style and digestive gland of the different mussel species is known, an estimate of the contribution digestive enzyme hydrolysis of natural detritus might make to the mussels' carbon budget can be made from the data. Total protein in the crystalline style and digestive gland of each of ten 60 mm shell length mussels of each species was measured according to the method of Lowry et al. (1951) and is shown in Table 30. Rates of reducing sugar release at 12,5^{o}C were taken from Table 24 for kelp and from Table 26 for detrital material. On the basis of a 42% carbon content for maltose, carbon production from kelp particulate matter by style enzymes, and from natural detrital material by style and digestive gland enzymes, is shown in Table 31. Carbon requirements of the mussels were

TABLE 30

Total protein content (+ SD) in the crystalline styles and digestive glands of C. meridionalis, M. galloprovincialis, P. perna and A. ater of 60 mm (+ 2 mm) shell length.

Mussel	Crystalline style protein	Digestive gland protein
C. meridionalis	5,48 (+ 0,05)	40,00 (+ 8,62)
M. galloprovincialis	6,34 (+ 0,69)	52,11 (+ 5,86)
P. perna	4,47 (+ 1,18)	23,15 (+ 4,60)
A. ater	4,09 (+ 0,99)	40,87 (+ 7,32)

TABLE 31: Potential carbon production from kelp and detrital substrates by style and digestive gland homogenates of C. meridionalis, M. galloprovincialis, P. perna and A. ater. Saccharogenesis at 12,5°C was converted to total carbon using total style and digestive gland protein estimates from Table 30. Respiration rates of 60 mm mussels were obtained from Griffiths (1980) for C. meridionalis, Griffiths and King (1979) for A. ater and Berry and Schleyer (1983) for P. perna. An estimate based on values of Bayne et al. (1984) for P. perna on the west coast was made for M. galloprovincialis respiration. Respiration rates were multiplied by a factor of 2 to allow for growth (Bayne and Newell, 1983) and carbon requirements were determined using a conversion of 1 ml O_2 = 0,530 mgC (Hawkins and Bayne, 1985). The percentage carbon requirements met by style and digestive gland hydrolysis of kelp and detrital substrates is shown, as well as the number of times per hour style and digestive gland protein would need to turn over to meet these requirements.

Enzyme	Saccharogenesis at 12,5°C mg maltose.mg protein.$^{-1}$h^{-1}		Protein content (mg)	mg Carbon.h^{-1}		Respiration at 12,5°C (ul O_2.h^{-1})	Carbon requirements (mg C.h^{-1})	% C requirements met by style and digestive gland hydrolysis		Style and digestive gland protein turnover.h^{-1} to support carbon requirements	
	kelp	detritus		kelp	detritus			kelp	detritus	kelp	detritus
C. meridionalis											
style	0,084	0,002	5,48	0,193	0,005	314	0,333	58	2	2	67
digestive gland		0,026	40,00		0,437				131		0,76
M. gallo-provincialis											
style	0,042	0,004	6,34	0,112	0,011	486	0,515	22	2	5	47
digestive gland		0,026	52,11		0,569				110		0,91
P. perna											
style	0,078	0,004	4,47	0,146	0,008	136	0,144	101	6	1	18
digestive gland		0,020	23,15		0,194				135		0,74
A. ater											
style	0,078	0,006	4,09	0,134	0,010	192	0,204	66	5	2	20
digestive gland		0,028	40,87		0,481				236		0,42

calculated from routine respiration rates for 60 mm shell length mussels of the different species. Respiration rates are given by Griffiths (1980a) for C. meridionalis, Griffiths and King (1979a) for A. ater and Berry and Schleyer (1983) for P. perna. Respiration rates of P. perna were corrected to 12,5°C using a temperature coefficient of 2,36 (Miller, quoted by Berry and Schleyer, 1983). Respiration rates for M. galloprovincialis are not known but Bayne et al. (1984) describe respiration rates for P. perna at Blouberg Strand. Since this species rarely occurs there and M. galloprovincialis has for many years been incorrectly identified as P. perna on the west coast of South Africa (Grant et al., 1984), an estimate of the respiration rates for M. galloprovincialis was made on the basis of the values of Bayne et al. (1984) for P. perna at Blouberg Strand (see also Chapters I and IV). Respiration rates were multiplied by a factor of 2 to allow for growth in field populations (Bayne and Newell, 1983), and carbon requirements of the mussels were determined using a respiratory coefficient of 1 ml O_2 = 0,530 mg C (Hawkins and Bayne, 1985)

It can be seen from Table 31 that on an hourly basis crystalline style hydrolysis of detrital material could supply between 2% and 6% of the mussels' carbon requirements. To satisfy the carbon requirements from this food source, the style protein would need to be renewed between 18 and 67 times an hour. Hydrolysis of kelp material by the style would supply between 22% and 101% of the mussels' carbon

requirements and style turnover rates would need to be between 1 and 5 times an hour to meet these requirements. Table 31 suggests that fresh kelp particulate material is a very much better food source than detrital material. Style turnover times shown in Table 31 differ considerably from turnover times estimated by Seiderer et al. (1982) of 24,4 hours for C. meridionalis, and 136,3 hours for P. perna, and those of between 30 and 111 hours shown in Chapter IV, Table 23. This indicates that caution must be exercised when constructing energy budgets from the enzymatic hydrolysis of substrates markedly different from those occurring in the natural environment.

Because of the greater protein content of the digestive gland, hydrolysis of detrital material could supply between 110% and 236% of the carbon requirements of the mussels (Table 31). With respect to hydrolytic potential, the digestive gland is therefore considerably more important than the style, and can release between 24 and 87 times more carbon. Thus carbon budgets and style turnover times such as those estimated for C. meridionalis and P. perna (Seiderer et al., 1982) and for C. virginica and G. demissa (Lucas and Newell, 1984), should not be calculated on the basis of style hydrolytic potential only.

However, even though Table 31 indicates that the mussels' carbon requirements can be met by hydrolysis of detrital material by the digestive gland, this would involve a protein

turnover time of approximately 2 hours, which is unrealistically rapid. It is not known how rapidly digestive gland protein is turned over and the digestive gland cells appear to be primarily organs of endocytic absorption and intracellular digestion (Morton, 1983). Sumner (1983) reported a very slow rate of production of new digestive gland cells in the mollusc Helix aspersa. It seems likely that bivalve digestive gland enzyme activity is related to a tidal or diurnal rythmn (Purchon, 1971; Morton, 1983) as is the crystalline style activity (Morton, 1956; Langton and Gabbott, 1974; Langton, 1977). Purchon (1971) suggests that the speed of replacement of digestive gland epithelium is very rapid. There appears to be a feeding related sequence of cytological changes in the digestive gland tubules and after absorption and intracellular digestion, digestive cells undergo a process of breakdown followed by regeneration (Morton, 1983). A major part of the cells making up the digestive gland epithelium may therefore be lost and renewed every 12 or 24 hours. If protein turnover occurred every 12 hours then digestive gland hydrolysis would supply between 9% and 20% of the mussels' carbon requirements.

Action of enzymes on phytoplankton cells

The action of mussel digestive enzymes on live phytoplankton cells likewise indicates that digestive gland extracts were more effective than style extracts at leaching pigments from cells (Table 29). It is also significant that the diatom species are more susceptible to attack by digestive enzymes

and microbial cellulase than the flagellates, in spite of the very thin cell membrane of the latter species. Dunaliella cells which have so often been used for absorption efficiency and clearance rate determinations, seem particularly resistant to the effects of digestive enzymes, in spite of their small size and consequent high surface area : volume ratio. Stuart (1982) also showed that Dunaliella cells were resistant to digestion by mussels. The resistance of live phytoplankton cells to digestive enzymes is surprising, since the cells are rapidly broken down during extracellular digestion in the gut (Figure 31 G, H and I). Purchon (1971) states that within 10 minutes of the addition of Isochrysis and Dunaliella cells to actively filtering Ostrea edulis, algal cells had been broken down by extracellular digestion in the stomach.

Rotation of the style against the gastric shield is poorly understood but may help to rupture cells, thus making the contents accessible to digestive enzymes. Although Kiorboe and Mohlenberg (1981) dispute this, generally it has been accepted that food enters the bivalve oesophagus in the form of a mucus string that is wound around the style which rotates against the gastric shield. This mechanical action of the style against the gastric shield helps to break up the food material (Bayne, 1976; Morton, 1983). Any inorganic particles incorporated in the mucus string would presumably help triturate the food to some extent, although Purchon (1971) thinks it is unlikely that sand grains are important

in this respect. Maceration in the gut would certainly improve the digestibility of refractory material and thin walled flagellates and delicate diatoms would only need to be brushed lightly against the gastric shield to bring about their rupture. The major problem with regard to investigating the role of style rotation in digestion is the impossibility of doing so without permanently damaging the organism.

CONCLUSIONS

Although style and digestive gland hydrolases are able to release carbon from a refractory detrital substrate, the digestive gland protein would need to be renewed at least once every 12 hours for these hydrolases to satisfy more than 20% of the mussels' carbon requirements. However, in Chapter I it was shown that mussels with absorption efficiencies of 40-61% could easily satisfy their carbon requirements from the particulate resource in the water column. In <u>in vitro</u> incubations, digestive enzymes can leach pigments from phytoplankton cells but no cell breakdown occurs. However, in the mussel gut considerable cell breakdown rapidly occurs. Thus <u>in vitro</u> incubations of digestive enzymes do not entirely duplicate processes in the mussel gut. Chapter IV demonstrated that there were distinct differences in the ability of style and digestive gland

enzymes of the four mussel species to hydrolyse purified substrates, and this might in part account for their differing distributions on the South African shore. Data presented in this Chapter shows that the interpretation of such differing enzyme activities is problematical, since naturally occurring detrital material is hydrolysed very slowly, and there appears to be little difference in the enzymatic capabilities of the different mussel species to digest such material.

The conditions of assay for reducing sugar release may be such that enzyme activity of style and digestive gland homogenates is limited, in spite of the apparent ease with which mussel digestive enzymes can hydrolyse substrates such as glycogen and laminarin. Slow rates of reducing sugar release may be a reflection of adverse assay conditions such as pH or osmotic concentrations and <u>in vitro</u> experiments may not be an effective indicator of the animals ability to utilize naturally occurring particulate material. The absorption of the organic component of particulate detrital material by bivalves may be partly dependent on the extracellular breakdown of such material by gut bacterial hydrolases or fermentative bacteria in the gut. If bacteria assist in breaking down detrital food particles during the bivalve digestive process, laboratory hydrolysis of the substrate may well be lower than in the gut of the animal. This question is examined in the next section.

CHAPTER VI

THE ROLE OF BACTERIA IN THE NUTRITION OF THE MUSSEL *MYTILUS GALLOPROVINCIALIS*

INTRODUCTION

The role that bacteria play in marine invertebrate nutrition has received considerable attention over the years but many questions remain to be answered. There is evidence that bacteria can be filtered from the water column by filter feeders such as tunicates, sponges and bivalves (Birkbeck and McHenery, 1982; Berry and Schleyer, 1983; Stuart and Klumpp, 1984; McHenery and Birkbeck,1985; Amouroux, 1986; Muir et al., 1986). Bivalves have been shown to be capable of lysing ingested bacteria (Birkbeck and McHenery, 1982; Seiderer et al., 1984; McHenery and Birkbeck, 1985; Muir et al., 1986), so that bacteria may form a component of their diet, particularly with respect to nitrogen metabolism. However, estimates of the importance of this food source vary from 30% of the hourly carbon demands (Muir et al., 1986) and all the nitrogen requirements (Seiderer et al., 1984) of C. meridionalis, to 5% of the carbon and approximately 10% of the nitrogen demands of M. edulis (Lucas et al., 1987)

In several species of temperate and tropical sea urchins, nitrogen fixing bacteria are present in the gut (Geurinot et al., 1977; Geurinot and Patriquin, 1981 a,b) and may fix as much as 139 µg N_2 per sea urchin per day (Geurinot et al., 1977). However the role that bacteria may play in the general digestive process of marine invertebrates is not clear. Lasker and Giese (1954) showed that sea urchins had a commensal gut microfloral population which could hydrolyse

agar, and echinoid gut bacteria were subsequently shown to be capable of hydrolysing certain algal polysaccharides (Prim and Lawrence, 1975). Fong and Mann (1980) showed that sea urchin gut microflora probably aid in the digestion of cellulose and can synthesize amino acids which then become available to the urchin. Crosby and Reid (1971) concluded that in Crassostrea gigas, gut microflora in conjunction with endogenous cellulases, played a significant role in the extracellular digestion of cellulose, but Payne et al. (1972) found no evidence of cellulolytic bacteria in Scrobicularia plana. More recently Wainwright and Mann (1982) demonstrated that the ability of mysids to digest cellulose depends largely on the gut microflora of the animal, and Waterbury et al. (1983) isolated a cellulose digesting bacterium from six species of Teredinid bivalve.

Bacterial populations residing in the gut of Mytilus edulis are different from the populations that exist in the water column, and may contain a high proportion of fermentative gram negative rods, as well as proteolytic and lypolytic forms (Prieur, 1981,1982). Unpublished data by Seiderer shows that during upwelling, 30% of bacterial isolates from the gut of C. meridionalis showed cellulase activity, while only 11% of isolates from sea water taken at the same time, demonstrated cellulolytic activity. Thus it would appear that gut bacteria may well be implicated in the digestive process of mytilids.

Chapter V has shown that *in vitro* hydrolysis of naturally occurring detritus by crystalline style and digestive gland homogenates of mussels of the South African coast could meet the carbon requirements of the animals, but would require very high turnover rates for the style and digestive gland protein. Assuming a digestive gland protein turnover time of 12 hours meant that 9-20% of the carbon requirememts could be met. It was also shown that live diatom and flagellate algal cells were not easily lysed by extracts of mussel digestive hydrolases. Bacteria in the mussel gut may assist in the initial breakdown of refractory natural food sources, and so aid digestion. The present work was carried out to determine whether the presence of bacteria in the mussel digestive tract influenced the absorption of organic material from detrital food sources.

MATERIALS AND METHODS

The approach adopted in these experiments was to feed detrital material to mussels and to compare the absorption efficiency obtained with that of mussels where the gut bacteria, and those bacteria attached to food particles, were suppressed by antibiotics.

Preparation of detrital food

Particulate material from the water column at Blouberg Strand was precipitated by centrifugation (9000 x g for 10 minutes). The pellet was resuspended in ammonium formate isotonic with sea water and again centrifuged at 9000 x g for 10 minutes. This was repeated twice, after which the pellet was lyophilized. To make up a stock solution for absorption efficiency experiments, approximately 30 g of this substrate was finely ground with a mortar and pestle, heated at 60°C for three hours to drive off any residual ammonium formate, and then resuspended and stirred in 500 ml of 0,45 µm filtered sea water for two hours. The solution was then passed successively through 105 µm, 45 µm and 20 µm sieves. Suitably diluted duplicate samples of the < 20 µm fraction were then counted with a model TA II Coulter Counter with a 70 µm aperture, to determine particle concentration. This was considered to be the equivalent of a natural detrital food solution. More than 90% of the particles were in the size range 1,6 µm - 5 µm.

Selection of mussels

M. galloprovincialis of 60 (±5) mm shell length were collected from the intertidal rocks at Blouberg Strand on the west coast of South Africa. Animals were maintained in glass aquaria with running seawater at 12,5(± 1)°C. The absorption efficiency of these animals was then determined in four experiments, using the method of Conover (1966).

Experimental procedure

All experiments were conducted in a constant temperature room at 12,5°C. Mussels were placed individually in 2 l beakers containing 1 l of 0,45 µm filtered sea water which was aerated and mixed by an airlift pump. An identical beaker containing an empty mussel shell was used as a control to monitor particle loss by sinking. Appropiate volumes of food stock solution were added to each beaker for a final concentration of 10×10^6 particles.l^{-1} (approximately 2 mg dry weight.l^{-1}) and this was controlled to within 20% of the desired concentration. Particle concentration was monitored with a model TA II Coulter Counter. Particle density was maintained by topping up on the basis of particle counts obtained. Baseline counts for filtered seawater were subtracted from each count. All faeces produced in the first four hours of the experiment were discarded. For the following five hours, faeces were collected from each beaker using a pasteur pipette. For each experiment, faeces and triplicate 6 ml volumes of food solution were filtered on to preashed, weighed 25 mm diameter GFC filters. Each filter paper was flushed with 5 ml ammonium formate isotonic with seawater, and duplicate ammonium formate blanks were obtained for each experiment. Filter papers were dried (60°C for three days), weighed, ashed at 450°C for eight hours and

reweighed. Absorption efficiency was calculated from the equation:

$$\frac{F - E}{(1 - E)F} \times 100$$

where F = the organic fraction of the food and

E = the organic fraction of the faeces (Conover, 1966).

Clearance rates were calculated from the standard equation:

$$\text{Clearance rate } (l.h^{-1}) = \frac{(\log_e N_1 - \log_e N_2 - a) \times V}{t}$$

where N_1 = particle concentration at time t_1;

N_2 = particle concentration at time t_2;

t = elapsed time in hours;

V = volume of suspension in litres;

a = rate of settlement of particles

$$= \frac{\log_e conc_o - \log_e conc_t}{t}$$

$conc_o$ = initial concentration of particles at time 0

$conc_t$ = concentration after time t in hours.

Experiment 1: Untreated mussels and non-sterile detritus

The purpose of this experiment was to determine the baseline absorption efficiency of mussels fed on a natural detrital substrate. The absorption efficiency of 12 untreated M. galloprovincialis was routinely determined as described

above, using non-sterile particulate suspension (10×10^6 particles.l^{-1}) as a food source. Bacterial counts of gut fluid and style homogenate from five animals were obtained as described below.

Experiment 2: Antibiotic treated mussels and sterile detritus

This experiment was performed to determine whether any change in the absorption efficiency of the M. galloprovincialis occurred when endogenous bacterial populations were suppressed and sterile detrital material was offered as a food source. Thirteen M. galloprovincialis were divided between two autoclaved beakers each containing 4 l of 0,2 µm filtered autoclaved seawater. Air was passed through sterile 0,2 µm filters affixed to sterile pipettes. Ampicillin (Boehringer Mannheim) and Streptomycin sulphate (Boehringer Mannheim) made up in sterile 0,2 µm filtered seawater were added to each beaker to a final concentration of 50 mg Ampicillin.l^{-1} and 25 mg Streptomycin.l^{-1}. After 12 hours and 20 hours the water in the beakers was replaced with fresh sterile antibiotic treated seawater. The mussels were fed every three hours on autoclaved detrital food stock made up as described previously. Six hours after the second water change, five mussels were sacrificed for bacterial counts of styles and gut fluid, and triplicate bacterial counts were made of the water in the 4 l beakers. For all mussel handling, sterile procedures were employed.

The remaining eight mussels were each transferred to an autoclaved 2 l glass beaker containing 1 l of 0,2 µm filtered seawater to which 50 mg Ampicillin and 25 mg Streptomycin were added. Bacterial counts were made of the water in 3 of the beakers. For this experiment all plastic components of the airlift pumps and piping were soaked in Biocide D for six hours, washed in boiling water and stored in 70% alcohol until use, when they were again thoroughly rinsed in boiling water. A sterile food solution was prepared from finely ground detrital material which was spread in a thin layer on a sterile glass tray and irradiated from a distance of 8 cm with ultraviolet light for 35 minutes. Trays were agitated every five minutes. Detrital stock solutions were then made up in 0,2 µm filtered seawater in sterile glassware and passed through a sterilised 20 µm sieve. This was added to experimental beakers to final concentration of 10×10^6 particles.l^{-1}. Triplicate bacterial counts of the stock solution were made. The absorption efficiency of the eight experimental mussels was determined as described above, except that beakers were kept covered and all additions of food and withdrawals for Coulter counting purposes were made with sterile pipettes. Bacterial counts in seawater were made for three of the beakers at the end of the experiment. After the experiment the eight mussels were again divided between two 4 l beakers.

Experiment 3: Mussels recolonised by bacteria, and non-sterile detritus

This experiment was performed to determine whether there was a change in the absorption efficiency once gut and style bacterial populations had been re-established in mussels that had been subjected to the antibiotic treatment described in Experiment 2. To each beaker containing 4 mussels from Experimnt 2, 4 l of seawater from the following sources was added: 2 l of seawater freshly collected from Blouberg Strand and 2 l of aquarium water into which was stirred approximately 50 mg of mussel faeces from animals kept in the aquarium. Mussel faeces were added to the water so that bacterial strains previously present in the gut might become re-established. Every three hours mussels were fed a non-sterile food solution prepared as previously described and aeration was as before but without the use of 0,2 µm filters. Triplicate bacterial counts were made of the water in the beakers. Water was changed every 12 hours. Animals were maintained in the beakers for 72 hours and the absorption efficiency was then routinely determined, using non-sterile food material, as described above. At the end of the experiment five mussels were sacrificed to determine style and gut fluid bacterial counts. Bacterial counts were made of the food stock solution.

Experiment 4: Untreated mussels and sterile detritus

The purpose of this experiment was to determine whether endogenous bacteria or bacteria attached to food particles caused the changes in absorption efficiencies that ocurred in Experiments 2 and 3. The absorption efficiency of untreated M. galloprovincialis that had been maintained in running sea water in the aquarium, was routinely determined as described above. However, the food material for this experiment was sterilised and prepared as described in Experiment 2.

Bacterial Counts

Bacterial counts of water and food solutions described above were made by plating triplicate 100 µl aliquots onto sea water agar plates (0,5% peptone, 0,1% yeast extract, 1,5% agar). This provided some indication of relative bacterial numbers in sea water, gut fluids and styles before and after treatments, although the percentage plateability can be very low (Kuznetsov et al., 1979). Five mussels were used to determine gut fluid and style bacterial numbers. Mussels were opened and rinsed with sterile sea water. The stomach was exposed and opened with sterile forceps and 5 µl of gut fluid was removed with a pipette. Gut fluids were pooled, thoroughly mixed with 2 ml sterile 20 mM phosphate buffer pH 6,9 containing 150 mM NaCl, and triplicate 100 µl aliquots were plated onto sea water agar plates. One ml of the remaining solution was diluted to 10 ml with phosphate buffer and a further triplicate 100 µl aliquots were plated.

Crystalline styles were removed, rinsed in sterile phosphate buffer and homogenized together in 5 ml phosphate buffer using a glass tissue grinder. Triplicate 100 µl aliquots were plated onto seawater agar plates. One ml of the remaining solution was diluted with phosphate buffer to 10 ml and a further triplicate of 100 µl aliquots were plated. All glassware was sterilised immediately before use. Plates were cultured at 22°C and colonies were counted after five days.

RESULTS

Bacterial counts for the experiments are presented in Table 32. The results of the absorption efficiency experiments as well as clearance rates and faecal production during the experiments are shown in Table 33.

Experiment 1: Bacterial numbers were $9,4 \times 10^5.ml^{-1}$ in the gut fluid and $7,5 \times 10^3.ml^{-1}$ in style homogenates of untreated mussels (Table 32). The baseline absorption efficiency of M. galloprovincialis fed on natural detrital material was 0,28 (Table 33). This is somewhat lower than values of 0,40 for C. meridionalis (Griffiths, 1980b) and 0,40 to 0,66 for C. meridionalis, P. perna and A. ater (Bayne et al., 1984) feeding on natural particulate material.

TABLE 32

Plateable bacteria.ml^{-1} (±SD) in filtered sea water and sea water in experimental beakers. Plateable bacterial numbers in the style and gut fluid of M. galloprovincialis before, during and after treatment with antibiotics are also shown.

	Bacterial concentration
Experiment 1	
0,45 µm filtered sea water	9,6 (±3,3) x 10^2 bacteria.ml^{-1}
Detrital food stock solution in 0,45 µm filtered sea water	8,2 (±1,4) x 10^4 bacteria.ml^{-1}
Untreated mussel gut fluid	9,4 (±0,2) x 10^5 bacteria.ml^{-1}
Untreated mussel style	7,5 (±0,8) x 10^3 bacteria.ml^{-1}
Experiment 2	
0,20 µm filtered sea water	3,2 (±1,2) x 10^2 bacteria.ml^{-1}
Antibiotic treated sea water in 4l beakers	20 (±26) bacteria.ml^{-1}
Antibiotic treated sea water in experimental beakers: Start of experiment	50 (±10) bacteria.ml^{-1}
Antibiotic treated sea water in experimental beakers: end of experiment	1,4 (±0,8) x 10^4 bacteria.ml^{-1}
UV sterilized food stock solution in 0,20 µm filtered sea water	2,9 (±0,9) x 10^3 bacteria.ml^{-1}
Antibiotic treated mussel gut fluid	5,0 (±1,2) x 10^3 bacteria.ml^{-1}
Antibiotic treated mussel style homogenate	1,7 (±0,1) x 10^2 bacteria.ml^{-1}
Experiment 3	
1:1 Aquarium sea water and freshly collected sea water	2,7 (±1,2) x 10^6 bacteria.ml^{-1}
Detrital food stock solution in 0,45 µm filtered sea water	10,6 (±2,3) x 10^4 bacteria.ml^{-1}
Recolonized mussel gut fluid	2,5 (±0,2) x 10^6 bacteria.ml^{-1}
Recolonized mussel style homogenate	4,5 (±0,9) x 10^3 bacteria.ml^{-1}
Experiment 4	
UV sterilised food stock solution in 0,20 µm filtered sea water	3,2 (±0,6) x 10^3 bacteria.ml^{-1}
Untreated mussel gut fluid	6,2 (±0,6) x 10^5 bacteria.ml^{-1}
Untreated mussel style homogenate	2,9 (±1,2) x 10^3 bacteria.ml^{-1}

TABLE 33

Clearance rates ($l.h^{-1}$), faecal production ($mg.animal^{-1}$) and absorption efficiencies, (±SD) of M. galloprovincialis before, during and after treatment with antibiotics. The organic content of the detrital food and the particle concentration in the experimental beakers is also shown. In the experiments mussels were treated with antibiotics and given sterile food. In Experiment 3 the same mussels were allowed to re-establish digestive system bacterial populations and were given non-sterile food. In Experiment 4 untreated mussels were given sterile food.

Treatment	Organic content	Particle conc particle.l^{-1}	Clearance rates $l.h^{-1}$	Faecal production $mg.animal^{-1}$	Absorption efficiency	n
1: No treatment	0,56 (±0,01)	10 x 10^6	2,91 (±0,52)	7,10 (±1,32)	0,28 (±0,08)	12
2: Antibiotic	0,57 (±0,01)	10 x 10^6	3,30 (±0,25)	9,26 (±1,22)	0,16 (±0,06)	8
3: Post-antibiotic	0,57 (±0,02)	10 x 10^6	3,20 (±1,07)	8,84 (±0,04)	0,37 (±0,04)	8
4: Sterile food	0,57 (±0,02)	10 x 10^6	1,56 (±0,48)	3,21 (±2,58)	0,16 (±0,08)	8

Experiment 2: Table 32 shows that antibiotic treated sea water in which mussels were maintained for 26 hours had a very low bacterial count (20 bacteria.ml^{-1}) when compared with bacterial counts from a sea water/aquarium water mixture (2,7 x 10^6 bacteria.ml^{-1}), 0,45 um filtered sea water (9,6 x 10^2 bacteria.ml^{-1}) and 0,2 µm filtered sea water (3,2 x 10^2 bacteria.ml^{-1}). At the start of the antibiotic treatment absorption efficiency experiment, 0,2 µm filtered sea water containing Ampicillin and Streptomycin had a bacterial count of 50 bacteria.ml^{-1} but this increased to 1,4 x 10^4 bacteria.ml^{-1} at the end of the experiment. This was probably a result of contamination caused by the necessary frequent removal of water for estimates of particle numbers, and by the addition of food stock solution. It is nevertheless surprising in view of the fact that Ampicillin and Streptomycin were present in each beaker. The mussels themselves may have contributed to the increase in bacterial numbers, since the gut fluids and styles of antibiotic treated mussels had higher counts than the antibiotic treated 0,2 µm filtered sea water at the start of the experiment (Table 32). The antibiotics may also not have been effective against all strains of bacteria. However, bacterial numbers present at the end of the experiment were only 0,5% of those in a mixture of aquarium and freshly collected sea water.

Ultraviolet sterilization of the detrital food source did not entirely eliminate bacteria from the stock solution, although bacterial numbers of sterilised detrital solutions were less

than 4% of untreated solutions. Many of the bacteria present in the ultraviolet treated solutions may have been associated with the 0,20 µm filtered sea water in which the stock solution was constituted, since this water contained $3,2 \times 10^2$ bacteria.ml^{-1} (Table 32).

Treatment by antibiotics reduced the number of bacteria in mussel gut fluid from $9,4 \times 10^5$ bacteria.ml^{-1} to 5×10^3 bacteria.ml^{-1} (Table 32). Antibiotic treatment did not reduce the number of style bacteria as dramatically. However, treated styles contained less than 6% of untreated style bacterial numbers. Microorganisms thickly coat mussel crystalline styles (Seiderer, pers comm.) and most are presumably killed by antibiotics. However, bacteria are also found embedded in the bivalve style matrix (Hameed and Paulpandian, 1985; Seiderer et al., 1987). To eliminate these, a complete style turnover in antibiotic treated water would be necessary and this presumably was not accomplished in the 26 hours that the mussels were exposed to the treatment. Antibiotic treated mussels had an absorption efficiency of 0,16 (Table 33). This was significantly different ($p < 0,01$, $t = 3,641$, 18 d. of f.) from the absorption efficiency of untreated animals given non-sterile food in Experiment 1.

Experiment 3: Bacterial counts in a sea water/aquarium mixture were $2,7 \times 10^6$.ml^{-1}. After 72 hours in this water, mussel gut fluid bacterial numbers increased from

$5,0 \times 10^3.ml^{-1}$ to $2,5 \times 10^6.ml^{-1}$, which was higher than bacterial numbers in untreated mussel gut fluid ($9,4 \times 10^5.ml^{-1}$, Table 32). Thus gut bacterial populations were effectively re-established in terms of numbers, although the population may not have had the same constitution. Bacterial recolonization of the style was not as complete and only 60% of the untreated style bacterial numbers were re-established (Table 32). Again, a complete style turnover would be necessary to ensure colonization of the style matrix. The absorption efficiency of animals with re-established gut and style bacterial populations increased from 0,16 to 0,37 when fed on non-sterile particulate material (Table 33). This was significantly different from the absorption efficiency after treatment with antibiotics ($p < 0,001$, $t = 8,076$, 14 d. of f.)

Experiment 4: Bacterial counts of untreated mussel style and gut fluid were slightly lower than those of Experiment 1 (Table 32). The absorption efficiency of these mussels fed on sterile detritus was 0,16, which was the same as that of the antibiotic treated mussels fed on sterile detritus (Table 33). This suggests that the suppression of bacterial populations associated with the particulate food source rather than the suppression of gut bacteria caused the decrease in absorption efficiency observed when antibiotic treated mussels were given sterile food (Experiment 2). However, the clearance rates and faecal production of the

mussels in Experiment 4 were very much lower than the other experiments (Table 33), which makes this result inconclusive.

Clearance rates of M. galloprovincialis during the course of the experiments are shown in Table 33. Untreated animals had a clearance rate of 2,91 $l.h^{-1}$. Animals in water with antibiotic additives had a clearance rate of 3,30 $l.h^{-1}$. The same animals had a clearance rate of 3,20 $l.h^{-1}$ after 72 hours in a mixture of aquarium water and fresh sea water. Faeces production during the five hour experimental period was similar in the first three experiments and ranged from 7,10 $mg.animal^{-1}$ to 9,26 $mg.animal^{-1}$ (Table 33). Organic content of the food ranged between 0,56 and 0,57 in the experiments.

DISCUSSION

The role of exogenous and endogenous bacteria in regulating absorption efficiency

The results presented here indicate that when the gut and style population of bacteria are reduced, the absorption efficiency of mussels is substantially reduced when sterile food is offered. However, since 16% of the organic component of the food could still be absorbed in the absence of gut microflora, it is evident that endogenous enzymes also play a part in the digestion of particulate material. When a gut

bacterial population was re-established, absorption efficiency in the same animals more than doubled (Tables 32 and 33). Gut fluid bacterial counts of untreated mussels were 45% of those animals' whose gut microfloral populations were first eliminated and then re-established. At the same time the absorption efficiency of these latter animals was 0,37 compared with 0,28 for untreated animals. Numbers of gut bacteria may thus have a direct bearing on the ability of the animal to digest particulate material.

However, Experiment 4 indicates that bacteria colonising the detrital food material rather than the gut bacterial population resulted in the increase in absorption efficiency observed when gut bacteria were re-established and a non-sterile food was offered (Experiment 3). There were however, considerable differences in the rates of clearance and faecal production between this experiment and the other three experiments (Table 33), which indicates some physiological disturbance in the mussels used in this last experiment. The reason for this is not clear, since the experimental conditions were exactly the same. Thus the results of this last experiment are unfortunately not conclusive. Stuart et al. (1982) showed that there was very little change in the absorption efficiency of Aulacomya ater fed on kelp particles, despite considerable variations in the microbial biomass attached to the particles. Thus changes in absorption efficiencies of M. galloprovincialis before and after antibiotic treatment can probably be ascribed in part

to changes in gut flora populations rather than to bacteria attached to the food particles.

The nature of the gut microfloral population may also influence the digestive capabilities of the animal. Mussels with absorption efficiencies of 0,28 were maintained in the aquarium for 72 hours prior to the experiment. Mussels with absorption efficiencies of 0,37 were maintained in a mixture of aquarium water and freshly collected sea water for 72 hours. Fresh sea water was collected in typical summer downwelling conditions. Under these conditions the bacterial population in the water column is highly diverse with a predominance of facultative anaerobes (Muir, 1986). The types of bacteria present in aquarium water have not been characterized. However it is likely that bacteria present during downwelling include types suited to digest the high particulate loads associated with these conditions (Chapter I). If these microbes are selectively retained in the mussel gut environment, animals with such a bacterial complement may be at a digestive advantage compared with animals with a gut flora composed primarily of bacteria from the aquarium environment. This might account for the higher absorption efficiencies in the third experiment compared with absorption efficiencies in the first experiment.

It is significant that Langdon and Siegfried (1984) found that growth of Crassostrea virginica was better when high bacterial numbers were present in culture conditions than

when bacterial numbers were low. From their work it cannot be determined whether this was a result of bacteria acting as a supplementary food source or because of improved breakdown and digestion of the food source by bacteria in the oyster gut. However, single bacterial cells are filtered inefficiently by C. virginica (Haven and Morales-Alamo, 1970; Sorokin, 1972) and the present work indicates that the increase in growth rates recorded by Langdon and Siegfried (1984) may be partly the result of improved breakdown of food particles by increased bacterial numbers in the oyster gut.

It is possible that the reduction in absorption efficiencies in M. galloprovincialis subjected to antibiotic treatment was a result of the response of the mussels to the antimicrobial substances, and was not linked to the presence or absence of a gut microflora. Langdon and Bolton (1984) noted a slight stimulation of filtering activity in oysters subjected to antibiotic and dispersant substances in the water, but over a two week period no toxic effects were observed. In the present experiment, mussels resumed filtering within a few minutes of being placed in the beakers with antibiotic additives. Clearance rates of animals in the antibiotic medium were slightly higher (3,30 $l.h^{-1}$) than those of animals which had not been treated with antibiotics (2,91 $l.h^{-1}$). However, the clearance rate of the same animals during and after treatment with antibiotics was 3,30 $l.h^{-1}$ compared with 3,20 $l.h^{-1}$ (Table 33). Thus there appears to be little difference in clearance rates with and

without antimicrobial additives (excluding Experiment 4). For the five hour experimental period, faeces production of antibiotic treated mussels was 9,26 mg.animal^{-1} compared with values of 7,10 mg.animal^{-1} for untreated animals and 8,84 mg.animal^{-1} for post-treatment animals (Table 33). Thus it is evident that the feeding activity of the mussels was not inhibited by the addition of the antimicrobial solution, and there is no indication that the mussels were unduly affected by the treatment.

Another possibility that must be considered is that mussels produce enzymes that are the only means of digestion of food particles, and the production of these enzymes is in some way inhibited by antibiotics. It is well known that the bivalve crystalline style and digestive gland produce enzymes capable of hydrolysing polysaccharide substrates (Kristensen, 1972a; Seiderer et al., 1982; Onishi et al., 1985; see Chapter IV), and in Experiment 2, mussels with very few gut and style bacteria absorbed 16% of the organic component of the food material. Thus it is conceivable that antibiotics could have inhibited enzyme production. However, there would appear to be a considerable delay between the production of enzymes which are incorporated in the style, and the use of those enzymes during digestion, since the style is a mucoprotein rod in which new material is added from the outside but mainly from the posterior end while middle lamellae leach out through the anterior end (Kristensen, 1972b). Mussel style turnover times measured by Seiderer et al. (1982) were

apparently linked to the hydrolytic activity of the style enzymes and the carbon requirements of the animal. The hydrolytic potential of M. galloprovincialis style enzymes is similar to that of P. perna, and in view of the hydrolytic potential by digestive gland enzymes (see Chapters IV and V), style turnover time is likely to be as long or longer for M. galloprovincialis than the 72-120 hours estimated for P. perna (Seiderer et al., 1982) Thus M. galloprovincialis subjected to 26 hours in a solution which inhibits enzyme production is probably utilizing pre-treatment style material for digestive purposes during the following nine hours, rather than new material. Therefore the digestive capabilities of the style at least, would be the same as those of pre-treatment animals. Equally, because of the slow nature of style turnover, style material laid down during the antibiotic treatment is likely to be available for use during an experiment 72 hours after Experiment 2. However absorption efficiencies increased from 0,16 to 0,37 in the later experiment. Thus for the style at least, it does not seem likely that changes in absorption efficiency after antibiotic treatment can be ascribed to inhibition of the production of endogenous enzymes.

Carbon budget

With data on filtration rates, food concentrations and weights, and the organic and carbon content of the food material, the extent to which the carbon intake of M. galloprovincialis is affected by different absorption

efficiencies can be calculated and is shown in Table 34. (Data from Experiment 4 has not been included since the filtration rates appear to be atypical). A comparison can also be made with carbon requirements supplied by in vitro enzyme hydrolysis of a similar food source. Total carbon available was calculated from the food concentration (2,335 mg.dw.l^{-1}), an organic fraction of 0,57 (Table 33) and a carbon fraction of 0,23 (Stenton-Dozey pers.comm.). Thus 0,306 mgC.l^{-1} is available to the animals and at a mean clearance rate (Experiments 1,2 and 3 only) of 3,14 $l.h^{-1}$ (Table 33), a total of 0,961 mgC.h^{-1} is ingested (Table 34). The actual carbon absorbed at the different absorption efficiencies can then be calculated. Carbon requirements can be estimated from respiration rates. No data are available for respiration rates of M. galloprovincialis so an estimate of the oxygen requirements was made from the values of Bayne et al. (1984) for P. perna at Blouberg Strand (see Chapters I, IV and V). Respiration rates were multiplied by a factor of two to allow for growth (Bayne and Newell, 1983) and carbon requirements in Table 34 were determined using a respiratory coefficient of 1 ml O_2 = 0,530 mg C (Hawkins and Bayne, 1985). The percentage of carbon requirements met by in vitro hydrolysis of natural detrital material was calculated from data in Table 31 (Chapter V), assuming a style and digestive gland protein turnover time of 12 hours.

Table 34 shows that a considerable proportion (52-69%) of the animal's carbon requirements can be met by digestion of a

TABLE 34

The percentage of M. galloprovincialis carbon requirements that are satisfied by absorption of natural detritus before, during and after treatment with antibiotics is compared with an estimate of the percentage of carbon requirements satisfied during in vitro enzyme assays (Section V, Table 31). Total carbon available was calculated from the food concentration (2,335 mg $dw.l^{-1}$) and organic fraction of 0,57 (Table 32), a carbon fraction of 0,23 (Stenton-Dozey, pers. comm) and clearance rates of 3,14 $l.h^{-1}$ (Table 32). The carbon requirements were estimated from respiration rates of P. perna at Blouberg Strand given by Bayne et al. (1984), multiplied by a factor of two to allow for growth (Bayne and Newell, 1983) and using a conversion of 1 ml O_2 = 0,530 mg C (Hawkins and Bayne, 1985). The percentage of carbon requirements met by in vitro enzyme assays was calculated from data in Table 30 assuming a 12 hourly turnover of style and digestive gland protein.

Treatment	Total Carbon available $mgC.h^{-1}$	Absorption efficiency	Carbon absorbed $mgC.h^{-1}$	Carbon requirements $mgC.h^{-1}$	% carbon requirements met	% carbon requirements from assays
1: No treatment	0,961	0,28	0,269	0,515	52	9
2: Antibiotic	0,961	0,16	0,154	0,515	30	9
3: Post-antibiotic	0,961	0,37	0,356	0,515	69	9

natural food source, when bacteria are present in the gut, style and food material. If only respiratory carbon requirements are considerd (i.e half the carbon requiremaents shown in Table 34) then the carbon demands for maintenance are met but there is little energy available for growth. When gut and style bacteria are removed and the food is sterile, 30% (60% of respiratory carbon demand) of the animal's carbon requirements can be met by endogenous enzyme hydrolysis of the particulate material. Assuming a style and digestive gland protein turnover time of 12 hours, only 9% of carbon requirements (18% of respiratory carbon demand) can by met by style and digestive gland carbohydrases under conditions of in vitro hydrolysis. Longer protein turnover times would mean that an even smaller proportion of the carbon requirements could be met.

CONCLUSIONS

When the endogenous bacterial population in the mussel digestive system is suppressed by antibiotics and the animals are fed a sterile food material, the absorption of organic material from the food declines significantly. When bacterial populations are re-established in the gut and crystalline style, and non-sterile food is offered, absorption efficiencies increase dramatically. Thus it appears that bacteria are implicated in the digestion of refractory

particulate material by mussels. It is not clear whether bacteria associated with the digestive system or those colonising the food particles result in the increase in absorption efficiency. Since bacteria appear to play a part in the digestive process of bivalves, caution must be exercised when attempting to estimate energy budgets such as those of Seiderer et al. (1984), Lucas and Newell (1984) and Chapters IV and V, from the results of in vitro enzyme assays. Furthermore, even without bacterial assistance in the breakdown of food, considerable discrepancies remain between estimates which are made from enzyme assays, of the carbon available to the animal, and those made from estimates of the carbon removed during passage through the digestive system.

GENERAL DISCUSSION

On the coast of South Africa, four species of mussel inhabit the rocky littoral and sublittoral zone. The black mussel C. meridionalis and the ribbed mussel A. ater are restricted to the low intertidal area and the subtidal kelp beds (Field et al., 1977; Grant et al., 1984), while the recently introduced (Grant and Cherry, 1985) M. galloprovincialis and the brown mussel P. perna are found in the mid and low intertidal area, as well as subtidally (Berry, 1978; Grant et al., 1984). C. meridionalis, M. galloprovincialis and A. ater are found on the west coast of South Africa, while P. perna is the dominant species on the south and east coasts but is seldom found west of the Cape Peninsula (Grant et al., 1984). M. galloprovincialis may be extending its range, since it has replaced C. meridionalis and A. ater as the dominant intertidal organism at certain localities on the west coast, and is apparently increasing in numbers on the coast east of the Cape Peninsula.

Growth rates of C. meridionalis on the west coast and P. perna on the east coast of South Africa are remarkably rapid, and these mussels can attain 60 mm in the their first year (Berry, 1978; Griffiths, 1981b), while M. galloprovincialis spat transplanted to culture ropes in Saldanha Bay can attain 60 mm in 10 months (Steyn, pers. comm.). Recently, rafts have been established at Port Elizabeth on the east coast and at Saldanha Bay on the west coast, for the culture of P. perna and M. galloprovincialis.

In addition, C. meridionalis and A. ater are major components in the diet of the commercially important rock lobster Jasus lalandii (Pollock, 1978; Griffiths and Seiderer, 1980). Thus the four mussel species all have a direct or indirect commercial value and factors affecting their growth are of considerable interest.

It is suggested that differences in both the geographic distribution of these mytilids and their ability to occupy different levels in the intertidal area, is in part related to their ability to efficiently digest the particulate food resource. The results presented in Chapter I showed that in the kelp bed area on the west coast of the Cape Peninsula, the carbon requirements of all four mussel species could easily be met by the particulate food resource in the water column, in spite of summer upwelling events which remove particulates from the area. However, the nitrogen requirements of C. meridionalis and M. galloprovincialis may be undersupplied at times. C:N ratios of 8,14 in summer and 9,90 in winter indicated that much of the particulate material was detritus, probably originating from the major macrophytes. Chapter II showed that seasonal differences occurred in the nature of the phytoplankton component of the particulate material. However, organic carbon : chlorophyll a ratios of 344 in summer and 742 in winter, as well as poor correlations between organic carbon and nitrogen, and chlorophyll a, confirmed that throughout the year, much of the particulate material in the water column is of a detrital

nature. Chapter III evaluated the methods commonly used to measure the release of reducing sugars from polysaccharide substrates by mussel enzymes, and Chapter IV investigated the major crystalline style and digestive gland carbohydrases that the four mytilid species use to digest particulate material filtered from the water column. Crystalline style saccharogenic rates were 10,51 mg maltose.mg protein.h^{-1} and 10,54 mg maltose.mg protein.h^{-1} for M. galloprovincialis and P. perna respectively, while C. meridionalis and A. ater had style saccharogenic rates of 4,62-6,54 mg maltose.mg protein.h^{-1}. The higher saccharogenic rates of M. galloprovincialis and P. perna would allow these two species to colonise rocks higher in the intertidal zone, where feeding times are limited. The lower rates of C. meridionalis and A. ater may restrict them to the lower intertidal area. Total saccharogenesis by the digestive gland of the west coast species C. meridionalis, M. galloprovincialis and A. ater is twice that of the south and east coast P. perna. It is suggested that the predominantly detrital food resources on the west coast require extensive processing in the digestive gland for effective hydrolysis. The low saccharogenic capabilities of P. perna's digestive gland prevent this species from successfully colonising the west coast, and limit it to a more easily digested phytoplankton dominated food supply on the south and east coasts. There appears to be some specialisation for a laminarin diet in the kelp bed mussels C. meridionalis and A. ater, since laminarinase activity

accounts for 57% of total style saccharogenesis compared with 38% for M. galloprovincialis and P. perna. M. galloprovincialis has more isozymes of α-amylase than the other mussel species, high temperature coefficients for both style and digestive gland α-amylase activity and a very much greater total hydrolytic potential than the other mussels. These factors may explain the recent success of M. galloprovincialis in colonising the west coast, and may allow it to spread to the east coast of South Africa. Differing style and digestive gland hydrolytic potentials among the four mussel species could therefore partly account for their differing intertidal and geographical distributions.

However, Chapter V showed that rates of style and digestive gland hydrolysis of naturally occurring detrital material by all four mussel species were similar and very slow. Also, phytoplankton cells were not easily lysed by digestive enzymes in in vitro incubations, although rapid lysis occurred in the mussel gut. The carbon requirements of the mussels could only be met from hydrolysis of detrital material by very rapid turnover rates of the style and digestive gland protein. Thus it is not easy to assess the importance of differing enzyme activities as a factor influencing the distributions of the mussel species. Chapter VI showed that the digestion of natural detrital material by mussels appears to be more than a process of bringing together enzymes and food material in the gut, and a

subsequent absorption of the products of hydrolysis. Endogenous bacteria or bacteria colonising the food particles assist in the digestion of refractory organic material, and in test tubes, lack of a suitable environment probably prevents these bacteria from performing their normal function in mytilid digestion.

It seems likely that a process of mechanical breakdown of food material is an important feature in mussel digestion and this is not duplicated in test tube incubations. Particulate material incubated in test tubes retains its particulate nature even after 20 hours incubation, whereas food material from the mussel gut soon becomes a liquid paste. This difference is particularly noticeable when algal cells are incubated with enzymes. In test tubes, Tetraselmis, Dunaliella and Thallassiosira cells maintain their identity even after 20 hours incubation with mussel digestive enzymes, whereas after 1-2 hours in the mussel gut, cells are extensively broken down and it is very difficult to recognise individual cells. Whether this breakdown of ingested material is the result of bacterial hydrolases or mechanical action or both is uncertain, but it occurs very rapidly and seems likely to be the effect of trituration of the food mass in the gut. Inorganic particles ingested with organic material may assist this process. The breakdown of particulate material would allow digestive enzymes to quickly come into contact with the cell contents and particularly the storage

products such as starch and laminarin which, in Chapters III and IV, have been shown to be rapidly hydrolysed by digestive tract carbohydrases. Differences in the enzyme activities of the bivalve species would then have a considerable effect in determining the efficiency of utilisation of a particular food source and therefore the distributions of species. There are however, other features of mussel physiology that might contribute to differences between the results of test tube incubations of substrates and digestive hydrolases, and the process of digestion in the mussel gut. There is evidence that the bivalve midgut may play a role in digestion (Reid, 1966; Payne et al., 1972; Mathers, 1973b) and enzyme activity has been found in the gastric shield of some bivalves (Kubomura, 1959; Halton and Owen, 1968). Furthermore, the gill epithelium displays α-amylase and protease activities which may start the digestive process long before the particulate food reaches the gut (Pequignat, 1973). In addition, in vitro incubations take no account of the apparently well developed ability of bivalves to select individual particles from a mixture (Kiorboe and Mohlenberg, 1981; Newell and Jordan, 1983; Pierson, 1983; Shumway et al., 1985). All of these features of mytilid nutrition will have an effect on the efficiency of utilisation of a particulate food resource and therefore affect the relative importance of the major carbohydrases in the digestive process.

An important consideration when examining carbohydrase enzyme activities as a mechanism controlling bivalve distribution,

is the relative importance of phytoplankton and macrophyte derived material compared with other sources of carbon and nitrogen gain, in the nutrition of the animals concerned. If mussels can utilise alternative methods of acquiring carbon and nitrogen from the environment, then their reliance on algal based food material will be less, and the importance of carbohydrate digestion in the total energy gain will be correspondingly less. Bacteria comprise such an additional food source, although they are relatively inefficiently retained by mussels (Stuart and Klumpp, 1984; Lucas et al., 1987) and their contribution to the carbon and nitrogen resources available to mussels in the kelp bed is likely to be less than 1% (see Chapter II). However, it is of interest in this respect that different mussel species appear to possess different lytic enzymes for digesting bacteria. The lysosome of the crystalline style of M. edulis is a true N-acetylmuramylhydrolase and it is always present in the style (McHenery and Birkbeck, 1982), whereas the activity of the lytic agent in the style of C. meridionalis is closely correlated with environmental temperature and, although it can lyse bacteria from the water column, it is not a true lysosyme (Seiderer et al., 1984; Muir et al., 1986). There is as yet no information on lysosymes in the styles of the mussels M. galloprovincialis, P. perna and A. ater, and this problem should be addressed in future work.

Although bacteria appear to make a small contribution to the nutritional requirements of mussels, the ability of these

organisms to rapidly take up dissolved organic matter (DOM) from very low ambient concentrations and against extreme concentration gradients (Pequignat, 1973; Manahan et al., 1982; Manahan, 1983; Manahan et al., 1983) may significantly reduce the importance of the digestion of particulate food material as a method of nutrient acquisition. Manahan et al. (1982) showed that M. edulis could remove 63%, 84% and 72% of aspartate, serine and glycine from natural sea water during a single passage of water through the mantle cavity. Further work by Manahan et al. (1983) showed that M. edulis can accumulate amino acids from ambient concentrations as low as 5 nM and against chemical gradients of approximately 10^6:1, and that between 37% and 75% of individual amino acids present at a concentration of 125 nM, are removed in a single passage through the mantle cavity. Cumulative rates of uptake of the amino acids were 4,9 $\mu M.gdw^{-1}.h^{-1}$.

There are no data on the free amino acid concentrations of sea water on the South African coast, but a rough estimate of the possible contribution of dissolved amino acids to filter feeder requirements in the kelp bed area can be made.

Newell et al. (1980a) calculated that 20,24 x 10^7 litres of mucilage is released annually by a 700 ha kelp bed of Ecklonia maxima and Laminaria pallida off the west coast of the Cape Peninsula. This amounted to 1458,38 x 10^4 kg dw of

mucilage per year. The Oudekraal kelp bed is approximately 25 ha. Assuming the same rate of mucilage production, 52,09 x 10^4 kg dw of mucilage is released annually. If this is released steadily throughout the year, 1427 kg of mucilage would then be released into the water column every day at Oudekraal. The kelp bed at Oudekraal is on average 10 m deep (Field et al., 1980). Thus there are approximately 2,5 x 10^9 litres of water in the kelp bed. The daily mucilage release would then be 571 $\mu g.l^{-1}$ of which approximately 5% consists of some 18 amino acids (Newell et al., 1980a). If the daily mucilage release occurred instantaneously, the free amino acid concentration from kelp mucilage would be 29 $\mu g.l^{-1}$. Assuming an average amino acid molecular weight of 100 (see Wright and Stevens, 1978; Manahan et al., 1983) the dissolved amino acid concentration would be 0,29 µM. This is probably a low estimate for these waters, since Manahan et al. (1983) recorded concentrations of 0,276 µM for serine, 0,223 µM for glycine and 0,078 µM for aspartate, for a cumulative value of 0,577 µM for these three amino acids alone, in the vicinity of a mussel bed. If the cumulative uptake rate for M. edulis of 4,9 $\mu M.gdw^{-1}.h^{-1}$ for a mixture of amino acids at a total concentration of 1,6 µM is the same for South African mussel species, the uptake rate from the 0,29 µM amino acid mixture derived from kelp mucilage is

$$\frac{0,29}{1,6} \times 4,9 = 0,888\ \mu M.gdw^{-1}.h^{-1}$$

Oxygen requirements for the mussels are approximately 0,4 ml $O_2.gdw^{-1}.h^{-1}$ (See Chapter I) and setting 1 mg mixed

amino acids equal to 1 ml O_2 for complete oxidation (Wright and Stevens, 1978; Manahan et al., 1983), energy requirements would be 4 $\mu M.gdw^{-1}.h^{-1}$. Thus the amino acids from mucilage could supply 22% of energy requirements based on oxidative metabolism. The potential for dissolved amino acids to contribute to the energy requirements of filter feeders is therefore considerable.

There is less quantitative information on the uptake of dissolved carbohydrates by filter feeders. Kelp mucilage contains approximately 6,5% free reducing sugars (Newell et al., 1980a), which would amount to $3,39 \times 10^4$ kg dw released annually into the water at Oudekraal. Considering the rapid uptake of these compounds by filter feeders (Pequignat, 1973), it is likely that they too contribute significantly towards the nutritional requirements of these animals. Thus the uptake of DOM warrants further investigation when quantifying filter feeder energy gains from the environment. However, the capture and digestion of particulate material are likely to be the most important processes in the acquisition of energy by mussels, particularly as they must compete for DOM with microorganisms which can also take up these compounds very rapidly (Linley et al., 1981; Lucas et al., 1981; Newell and Lucas, 1981).

Perhaps the most important factor involved in assessing the saccharogenic potential of the style and digestive gland enzymes of different mussel species is the rate at which the

crystalline style and digestive gland can supply enzyme protein for digestion, as this will greatly affect calculations of hydrolytic potential. A rapid rate of enzyme production may compensate for a low hydrolytic potential. In Chapters IV and V the rate at which the style and digestive gland protein would need to be renewed to meet the carbon requirements of the animals has been calculated on the basis of the carbon released by the whole style or digestive gland (measured as total protein content). However, a very much better assessment of the potential for hydrolysis by the mussel digestive system would be obtained if the rates of style and digestive gland enzyme production were known, since this may vary in different species. There have been very few investigations into this process.

Edmonson (1920) estimated the time for complete style renewal in Mya arenaria was 74 days. The only recent attempt to measure style turnover rates in mussels was by Seiderer et al. (1982), in which style protein appeared to be renewed approximately every 18 hours in C. meridionalis and every 72 hours in P. perna. There are no data available on rates of protein renewal in the digestive gland. Experiments on the effect of starvation on style enzyme activities have shown that very rapid changes can occur in the crystalline style and these changes are not the same for all mussel species. In starved (10 days) mussels, the style generally became smaller in relation to total body weight. When starved mussels were fed a suspension of Phaeodactylum, within 3 hours styles of

A. ater often dissolved to a liquid jelly, while those of C. meridionalis and M. galloprovincialis increased in size and were back to their normal size after 24 hours. However M. galloprovincialis styles became very much softer and more elastic during this period, although it is not known if this was the result of new material being laid down. A. ater styles reappeared within about 12 hours of the start of feeding. When starved C. meridionalis were fed, the protein content as a percentage of the dry weight of style declined very rapidly in the first 6 hours after feeding, while α-amylase activity per unit protein increased. In A. ater, the protein content as a percentage of the style dry weight increased, while α-amylase activity per unit protein declined dramatically (Fielding, unpublished data). Thus there would appear to be a difference in the priority in which style proteins are manufactured in the two species, and style dissolution and manufacture may be a complex process. More detailed knowledge is required to obtain a better understanding of the role of this organ in the digestive process.

In conclusion, on the basis of their ability to hydrolyse commercially pure polysaccharide substrates, four South African mussel species exhibit considerable differences in the hydrolytic potentials of the major polysaccharases present in the crystalline style and digestive gland. These differences in polysaccharase activity indicate differences in their abilities to utilise food resources and this could

partly account for their distributions along the coastline. However, solutions of style and digestive gland enzymes do not easily hydrolyse detrital material and it is not clear how important such differences in polysaccharase activities are in the digestion of naturally occurring particulates. Particular attention should be given to determining the extent of mechanical and bacterial breakdown of food in the mussel gut and the process of style renewal.

REFERENCES.

AGNISOLA, C., S. SALVADORE & V. SEARDI, 1981. On the occurence of cellulolytic activity in the digestive gland of some marine carnivorous molluscs. Comp. Biochem. Physiol. 70B : 521-525.

ALEMANY, M. & M. ROSELL-PEREZ, 1973. Two different amylase activities in the sea mussel, Mytilus edulis L.. Rev. Esp. Fisiol. 29 : 217-222.

ALLENDORF, P., 1981. Experiments to determine the relevance of dissolved amino acids for the nutrition of marine benthic animals. Kieler. Meeresforsch. Sonderh. 5 : 557-565

AMOUROUX, J.M., 1986. Comparative study of the carbon cycle in Venus verrucose fed on bacteria and phytoplankton I. Consumption of bacteria (Lactobacillus sp.). Mar. Biol. 90 : 237-241.

ANDREWS, W.R.H. & D.L. CRAM, 1969. Combined serial shipboard upwelling study in the Benguela current. Nature 24 : 902-904.

ANDREWS, W.R.H. & L. HUTCHINGS, 1980. Upwelling in the southern Benguela current. Prog. Oceanog. 9 : 1-81.

BACON, E.J., 1984. The analysis of marine algal photosynthetic pigments by High Performance Liquid Chromatography. M.Sc. Thesis, University of Cape Town, Rondebosch, S.A.

BANG, N.D., 1973. The southern Benguela system: finer oceanic structure and atmospheric determinants. (An exercise in the exploitation of elementary research resources). PhD. thesis, University of Cape Town, Rondebosch, S.A..

BANSE, K., 1977. Determining the carbon to chlorophyll ratio of natural phytoplankton. Mar. Biol. 41 : 199-212.

BARLOW, R.G., 1982. Phytoplankton ecology in the southern Benguela current. I. Biochemical composition. J. Exp. Mar. Biol. Ecol. 63 : 209-227.

BARRET, J. & S.W. JEFFREY, 1964. Chlorophyllase and formation of a typical chlorophyllide in marine algae. Plant Physiol. 39 : 44-47.

BAYNE, B.L., 1976 (ed). Marine mussels: their ecology and physiology. Cambridge University Press. London 506pp.

BAYNE, B.L. & J. WIDDOWS, 1978. The physiological ecology of two populations of Mytilus edulis L. Oecologia 37(2) : 137-162.

BAYNE, B.L. & R.C. NEWELL, 1983. Physiological energetics of marine molluscs. In: A.S.M. Salueddin and K.M. Wilbur (eds). The Mollusca, vol 4. Physiology, Part I, pp 407-515. Academic Press, New York.

BAYNE, B.L., D.W. KLUMPP & K.R. CLARKE, 1984. Aspects of feeding, including estimates of gut residence time, in three mytilid species (Bivalvia, Mollusca) at two contrasting sites in the Cape Peninsula, South Africa. Oecologia 64 : 26-33.

BERNFELD, P.C., 1955. Amylases α and β. In: S.P. Colwick & N.P. Kaplan (eds). Methods in Enzymology, Vol 1, pp 148-150. Academic Press, New York.

BERRY, P.F., 1978. Reproduction, growth and production in the mussel, Perna perna (Linnaeus), on the east coast of South Africa. Invest. Rep. oceanogr. Res. Inst., Durban. 48 : 1-28.

BERRY, P.F. & M.H. SCHLEYER, 1983. The brown mussel Perna perna on the Natal coast, South Africa: utilization of available food and energy budget. Mar. Ecol. Prog. Ser. 13 : 201-210.

BEZUKLADNIKOV, P.W. & L.A. ELYAKOVA, 1986. A study of the pattern of action of endo-(1-3)-β-D-glucanases from marine bivalves on a polymer substrate labelled at the reducing end. Carbohydr. Res. 152 : 261-273.

BIRKBECK, T.H. & J.G. McHENERY, 1982. Degradation of bacteria by Mytilus edulis. Mar. Biol. 72 : 7-18.

BIRKBECK, T.H. & J.G. McHENERY, 1984. Chitinase in the mussel Mytilus edulis (L.). Comp. Biochem. Physiol. 77B(4) : 861-865.

BISHOP J.K.B., D.R. KETTEN & J.M. EDMOND, 1978. The chemistry, biology, and vertical flux of particulate matter from the upper 400m of the Cape Basin in the Southeast Atlantic ocean. Deep Sea Res. 25 : 1121-1161.

BORCHERS, P. & J.G. FIELD, 1981. The effect of kelp shading on phytoplankton production. Bot. Mar. 24 : 89-91.

BOWLES, M.D., H.W. PAERL & J. TUCKER, 1985. Effective solvents and extraction periods employed in phytoplankton carotenoid and chlorophyll determinations. Can. J. Fish. Aquat. Sci. 42 : 1127-1131.

BRAUMANN, T. & L.H. GRIMME, 1981. Reverse phase Liquid chromatography of chlorophylls and carotenoids. Biochim. Biophys. Acta. 637 : 8-17.

BREUIL, C. & J.N. SADDLER, 1985. Comparison of the 3,5-dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity. Enzyme and Microb. Technol. 7 : 327-332.

BROWN, L.M., B.T. HARGRAVE & M.D. MACKINNON, 1981. Analysis of chlorophyll a in sediments by high-pressure liquid chromatography. Can. J. Fish. Aquat. Sci. 38 : 205-214.

BROWN, P.C., 1981. Pelagic phytoplankton, primary production and nutrient supply in the southern Benguela region. Trans. R. Soc. S. Afr. 44 : 347-356.

BROWN, P.C., 1984. Primary production at two contrasting nearshore sites in the Southern Benguela upwelling region, 1977-1979. S. Afr. J. Mar. Sci. 2 : 205-227.

BROWN, P.C. & J.G. FIELD, 1985. Diel variation in production rates of natural phytoplankton populations in the southern Benguela upwelling regions. Bot. Mar. 28 : 201-208.

BROWN, P.C. & J.G. FIELD, 1986. Factors limiting phytoplankton production in a nearshore upwelling area. J. Plank. Res. 8(1) : 55-68.

BULL, A.T. & C.G.C. CHESTERS, 1966. The biochemistry of laminarin and the nature of laminarinase. Adv. Enzymol. 28 : 325-364.

CABEZAS, J.A., A. REGLERO, A. DE PEDRO, T. DIEZ & P. CALVO, 1981. Hydrolysis of natural and synthetic substrates by α-L-fucosidase, β-D-glucuronidase and β-N-acetylhexos-aminidase purified from molluscs. Int. J. Biochem. 13 : 389-393.

CARTER, R.A., 1982. Phytoplankton biomass and production in a southern Benguela kelp bed system. Mar. Ecol. Prog. Ser. 8 : 9-14.

CHESTERS, C.G.C. and A.T. BULL, 1963. The enzymic degradation of laminarin. Biochem. J. 86 : 28-46.

CLIFF, G., 1979. The contribution by phytoplankton, bacteria and detritus to a rocky shore ecosystem. M.Sc. Thesis. University of Cape Town, Rondebosch, South Africa.

CONOVER, R.J., 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11 : 338-345.

COUPIN, H., 1900. Sur les fonchons de la Tige Cristilline des Acephates. C.R. Acad. Sci. Paris 130 : 1214-1216.

CROSBY, N.D. & G.B. REID, 1971. Relationships between food, phylogeny, and cellulose digestion in the Bivalvia. Can. J. Zool. 49 : 617- 622.

DALEY, F.J., C.B.J. GRAY & S.R. BROWN, 1973. A quantitative, semi-routine method for determining algal and sedimentary chlorophyll derivatives. J. Fish. Res. Bd. Can. 30 (3) : 345-356.

DIECKMANN, G.S., 1978. Aspects of growth and production of Laminaria pallida (Grev.) off the Cape Peninsula. MSc. Thesis. Department of Botany, University of Cape Town, Rondebosch, S.A..

DIECKMANN, G.S., 1980. Aspects of the ecology of Laminaria pallida (Grev.) J.Ag. off the Cape Peninsula (South Africa). Bot. Mar. 23 :579-585.

DIEM, K. & C. LENTNER, 1970. (Eds): Scientific Tables, J.R. Geigy, Switzerland, seventh edition, 809 pp.

EDMONSON, C.H. (1920). The reformation of the crystalline style in Mya arenaria after extraction. J. Exp. Zool. 30 : 259-291.

ELYAKOVA, L.A., 1972. Distribution of cellulases and chitinases in marine invertebrates. Comp. Biochem. Physiol. 43B : 67-70.

ELYAKOVA, L.A. & T.N. ZVYAGINTSEVA, 1981. Kinetic peculiarities in the action of 1,3, β glucanases from different sources. Bioorg Khim 7(5) : 680-685.

FAROUKI, E. & D.J. GUNN, 1983. Some effects of metal ions on the estimation of reducing sugars in biological media. Biotechnol. Bioeng. 25 : 1905-1911.

FIELD, J.G., N. JARMAN, G. DIECKMANN, C.L. GRIFFITHS, B. VELIMIROV & P. ZOUTENDYK, 1977. The ecology of kelp bed communities in the Benguela upwelling system. Analysis of biomass and spatial distribution. Helgolander wiss. Meeresunters 30 : 495-518.

FIELD, J.G., C.L. GRIFFITHS & E.A. LINLEY, 1980. Upwelling in a nearshore marine ecosystem and its biological implications. Estuar. coast. mar. Sci. 11 : 133-150.

FONG, W. & K.H. MANN, 1980. Role of gut flora in the transfer of amino acids through a marine food chain. Can. J. Fish. Aquat. Sci. 37 : 88-96.

FOULDS, J.B. & K.H. MANN, 1978. Cellulose digestion in Mysis stenolepsis and its ecological implications. Limnol. Oceanogr. 23(4) : 760-766.

FRECHETTE, M. & E. BOURGET, 1985. Energy flow between the pelagic and benthic zones: Factors controlling particulate organic matter available to an intertidal mussel bed. Can. J. Fish. Aquat. Sci. 42 : 1158-1165.

GABBOTT, P.A., 1983. Developmental and seasonal metabolic activities in marine molluscs. In: P.W. Hochachka (ed). The Mollusca, vol 2, Environmental Biochemistry and Physiology, pp 165-217. Academic Press, New York.

GALLI, D.R. & A.C. GIESE, 1959. Carbohydrate digestion in a herbivorous snail, Tegula finebralis. J. Exp. Zool. 140 : 415-440.

GARDINER, A., 1980. Adjustment of the activity of α-amylase extracted from the style of the ribbed mussel, Aulacomya ater in response to thermal acclimation and starvation. Honours project, University of Cape Town.

GARSIDE, C. & J.P. RILEY, 1969. A thin-layer chromatographic method for the determination of plant pigments in sea water and cultures. Anal. Chim. Acta. 46 : 179-191.

GEURINOT, M.L. & D.G. PATRIQUIN, 1981a. N_2-fixing vibrios isolated from the gastrointestinal tract of sea urchins. Can. J. Microbiol. 27 : 311-317.

GEURINOT, M.L. & D.G. PATRIQUIN, 1981b. The association of N_2-fixing bacteria with sea urchins. Mar. Biol. 62 : 197-207.

GEURINOT, M.L., W. FONG & D.G. PATRIQUIN, 1977. Nitrogen fixation (acetylene reduction) associated with sea urchins (Strongylocentrotus droebachiensis) feeding on seaweeds and eelgrass. J. Fish. Res. Bd. Can. 34 : 416-420.

GIANFREDA, L., A. IMPERATO, R. PALESCANDOLA, & V. SCARDI, 1979. Distribution of C-1,4-glucanase and C-glucoside activities among marine molluscs with different feeding habits. Comp. Biochem. Physiol. 63B : 345-348.

GIESKES, W.W.C., G.W. KRAAY & S.B. TIJSSEN, 1978. Chlorophylls and their degradation products in the deep pigment maximum layer of the tropical north Atlantic. Neth. J. Sea. Res. 12 : 195-204.

GRANT, W.S. & M.I. CHERRY, 1985. Mytilus galloprovincialis Lmk. in southern Africa. J. Exp. Mar. Biol. Ecol. 90 :179-191.

GRANT, W.S., M.I. CHERRY & A.T. LOMBARD, 1984. A cryptic species of Mytilus (Mollusca: Bivalvia) on the west coast of South Africa. S. Afr. J. mar. Sci. 2 : 149-162.

GREENWOOD, P.J., 1980. Growth, respiration and tentative energy budgets for two populations of the sea urchin Parenchinus angulosus (Leske). Estuar. coastal. mar. Sci. 10 : 347-367.

GRIFFITHS, C.L. & J.A. KING, 1979a. Some relationships between size, food availability and energy balance in the ribbed mussel Aulacomya ater. Mar. Biol. 51 : 141-149.

GRIFFITHS, C.L. & J.A. KING, 1979b. Energy expended on growth and gonad output in the ribbed mussel Aulacomya ater. Mar. Biol. 53 : 217-222.

GRIFFITHS, C.L. & L.J. SEIDERER, 1980. Rock-lobsters and mussels - limitations and preferences in a predator-prey interaction. J. Exp. Mar. Biol. Ecol. 44: 95-109.

GRIFFITHS, C.L., 1981a. Grazers and filter feeders in the nearshore zone of the southern Benguela. Trans. R. Soc. S. Afr. 44(3) : 335-340.

GRIFFITHS, R.J., 1977. Reproductive cycles in littoral populations of Choromytilus meridionalis (Kr) and Aulacomya ater (Molina) with a quantitative assessment of gamete production in the former. J. Exp. Mar. Bio. Ecol. 30 : 53-71.

HART, H. & R.D. SCHUETZ, 1966. Organic Chemistry, Houghton Mifflin Company, Boston, third edition, 335 pp.

HAVEN, D.S. & R. MORALES-ALAMO, 1970. Filtration of particles from suspension by the American oyster Crassostrea virginica. Biol. Bull. (Woods Hole, Mass.) 139: 246-264.

HAWKINS, A.J.S., 1983. Metabolic strategy in the marine mussel Mytilus edulis L. PhD Thesis, University of Exeter.

HAWKINS, A.J.S., B.L. BAYNE & K.R. CLARKE, 1983. Coordinated rythms of digestion, absorption and excretion in Mytilus edulis (Bivalvia: Mollusca). Mar. Biol. 74 : 41-48.

HAWKINS, A.J.S. & B.L. BAYNE, 1984. Seasonal variation in the balance between physiological mechanisms of feeding and digestion in Mytilus edulis (Bivalvia:Mollusca). Mar. Biol. 82 : 233-240.

HAWKINS, A.J.S., 1985. Relationships between the synthesis and breakdown of protein, dietary absorption and turnovers of nitrogen and carbon in the blue mussel, Mytilus edulis L. Oecologia 66 : 42-49.

HAWKINS, A.J.S. & B.L. BAYNE, 1985. Seasonal variation in the relative utilization of carbon and nitrogen by the mussel Mytilus edulis: budgets, conversion efficiencies and maintenance requirements. Mar. Ecol. Prog. Ser. 25 : 181-188.

HAWKINS, A.J.S., B.L. BAYNE, R.F.C. MANTOURA & C.A. LLEWELLYN, 1986. Chlorophyll degradation and absorption throughout the digestive system of the blue mussel Mytilus edulis L. J. Exp. Mar. Biol. Ecol.

HOCHACHKA, P.W. & G.N. SOMERO, 1973. Strategies of biochemical adaptation. W.B. Saunders Company Philadelphia, pp 179-270.

HOFFMAN, K.H., 1983. Metabolic and enzyme adaptation to temperature and pressure. In: P.W. Hochachka (ed). The Mollusca. Vol 2, Environmental Biochemistry and Physiology pp 220-255.

HOLDEN, M., 1962. Separation by paper chromatography of chlorophylls a and b and some of their breakdown products. Biochim. Biophys. Acta. 56 : 378-379.

HOLM-HANSEN, O., 1978. Chlorophyll a determination : improvements in methodology. Oikos 30 : 438-447.

HOLM-HANSEN, O., C.J. LORENZEN, R.W. HOLMES & J.D.H. STRICKLAND, 1965. Fluorometric determination of chlorophyll. J. Cons. perm. int. Explor. Mer. 39 : 3-15.

HORIUCHI, S. & C.E. LANE, 1965. Digestive enzymes of the crystalline style of Strombus gigas Linne. I. Cellulase and some other carbohydrases. Biol. Bull. (Woods Hole, Mass.), 129 : 273-281.

HORIUCHI, S. & C.E. LANE, 1966. Carbohydrases of the crystalline style and hepatopancreas of Strombus gigas Linne. Comp. Biochem. Physiol. 17 : 1189-1197.

JACOBER, L.F., C. RICE & A.G. RAND, 1980. Characterization of the carbohydrase degrading enzymes in the surf clam crystalline style. J. Food Sci. 45 : 381-385.

JACOBSEN, T.R., 1978. A quantitative method for the separation of chlorophylls a and b from phytoplankton pigments by high pressure liquid chromatography. Mar. Sci. Comm. 4 : 33-47.

JACOBSEN, T.R., 1982. Comparison of chlorophyll a measurements by fluorometric, spectrophotometric and high pressure liquid chromatographic methods in aquatic environments. Archiv. fuer Hydrobiologie Beih. 16 : 35-45.

JARMAN, N.G. & R.A. CARTER, 1981. The primary producers of the inshore regions of the Benguela. Trans. R. Soc. Afr. 44(3) : 321-325.

JEFFREY, S.W., 1968. Quantitative thin-layer chromatography of chlorophylls and carotenoids from marine algae. Biochim. Biophys. Acta. 162 : 271-285.

JEFFREY, S.W., 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Mar. Biol. 26 : 101-110.

JEFFREY, S.W. & G.F. HUMPHREY, 1975. New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae, and natural phytoplankton. Biochem. Physol. Pflanzen. 167: 191-194.

JEFFREY, S.W., 1981. An improved thin-layer chromatographic technique for marine phytoplankton pigments. Limnol. Oceanogr. 26(1) : 191-197.

JENSEN, A. & E. SAKSHAUG, 1973. Studies on the phytoplankton ecology of the Trondheimsfijord. II. Chloroplast pigments in relation to abundance and physiological state of the phytoplankton. J. Exp. Mar. Biol. Ecol. 11 : 137-155.

JENSEN, A., 1978. Chlorophylls and carotenoids. In: J.A. Hellebust and J.S. Craigie (eds.), Handbook of Phycological Methods 2. pp 59-71. Cambridge Univ. Press, New York.

JENSEN, L.M., 1983. Phytoplankton release of extracellular organic carbon, molecular weight composition, and bacterial composition. Mar. Ecol. Prog. Ser. 11 : 39-48.

JURY, M.R., 1980. Characteristics of summer wind field and air sea interactions over the Cape Peninsula upwelling regions. MSc. Thesis. University of Cape Town. South Africa. 131pp.

KIORBOE, T. & F. MOHLENBERG, 1981. Particle selection in suspension-feeding bivalves. Mar. Ecol. Prog. Ser. 5 : 291-296.

KLUMPP, D.W., 1984. Nutritional ecology of the ascidian Pyura stolonifera: influence of body size, food quantity and quality on filter feeding, respiration, assimilation efficiency and energy balance. Mar Ecol. Prog. Ser. 19 : 269-284.

KOOP, K., R.C. NEWELL, M.I. LUCAS, 1982. Biodegradation and carbon flow based on kelp (Eklonia maxima) debris in a sandy beach microcosm. Mar. Ecol. Prog. Ser. 7 : 315-326

KOOPMANS, J.J.C., 1970. Cellulases in Molluscs. I. The nature of the cellulases in Helix pomata and Cardium edule. Neth. J. Zool. 20(4) :445-463.

KRISTENSEN, J.H., 1972a. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurance of the carbohydrates studied. Mar. Biol. 14 : 130-142.

KRISTENSEN, J.H., 1972b. Structure and function of crystalline styles of bivalves. Ophelia 10 : 91-108.

KUBOMURA, K., 1959. The gastric shield of the bivalve mollusk Meretrix meretric. Sci. Rep. Saitama Univ. Ser. B 3 : 153-159.

KUEH, C.S.W. & K. CHAN, 1985. Bacteria in bivalve shellfish with special reference to the oyster. J. Appl. Bact. 59 : 41-47.

KUZNETSOV, S.I., G.A. DUBININA & N.A. LAPTERA, 1979. Biology of oligotrophic bacteria. Ann. Rev. Micro. 33 : 377-387.

LANGDON C.J. & E.T. BOLTON, 1984. A microparticulate diet for a suspension-feeding bivalve mollusc, Crassostrea virginica (Gmelin). J. Exp. Mar. Biol. Ecol. 82 : 239-258.

LANGDON, C.J. & C.A. SIEGFRIED, 1984. Progress in the development of artificial diets for bivalve filter feeders. Aquaculture 39 : 135-153.

LANGTON, R.W. & P.A. GABBOTT, 1974. The tidal rythm of extracellular digestion and the response to feeding in Ostrea edulis. Mar. Biol. 24 : 181-187.

LANGTON, R.W., 1977. Digestive rythms in the mussel Mytilus edulis. Mar. Biol. 41 : 53-58.

LASKER, R. & A.C. GIESE, 1954. Nutrition of the sea urchin Strongylocentrotus purpuratus. Biol. Bull. mar. biol. Lab., Woods Hole 106 : 328-340.

LAVINE, T.F., 1946. A study of the enzymatic and other properties of the crystalline style of clams: evidence for the presence of cellulase. J. Cell. Comp. Physiol. 28 : 183-195.

LEHMAN, P.W., 1981. Comparison of chlorophyll a and carotenoid pigments as predictors of phytoplankton biomass. Mar. Biol. 65 : 237-244.

LINDLEY, M.G., R.S. SHALLENBERGER & S.M. HERBERT, 1976. Purification and characterization of a molluscan β-D-(1-3)-glucan hydrolase. Fd. Chem. 1 : 149.

LINLEY, E.A.S., R.C. NEWELL, 1981. Microheterotrophic communities associated with the degradation of kelp debris. Kieler Meeresforsch. Sonderh. 5 : 345-355.

LINLEY, E.A.S., R.C. NEWELL & S.A. BOSMA, 1981. Heterotrophic utilization of mucilage released during fragmentation of kelp (Ecklonia maxima and Laminaria pallida). I. Development of microbial communities associated with the degradation of kelp mucilage. Mar. Ecol. Prog. Ser. 4 : 31-41.

LINLEY, E.A.S. & J.G. FIELD, 1982. The nature and ecological significance of bacterial aggregation in a nearshore upwelling system. Estuar. Coast. Shelf Sci. 14 : 1-11.

LORENZEN, C.J., 1967. Determination of chlorophyll and phaeopigments: Spectrophotometric equations. Limnol. Oceanogr. 12 : 243-246.

LORENZEN, C.J., 1968. Carbon-chlorophyll relationships in an upwelling area. Limnol. Oceanogr. 13 : 202-204.

LORENZEN, C.J. & S.W. JEFFREY, 1980. Determination of chlorophyll in sea water. : Report of intercalibration tests. Unesco technical papers in marine science No. 35. 19pp,

LOWRY, O.H., N.J. ROSEBROUGH, A. LEWIS FARR, & R.J. RANDALL, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 : 265-275.

LUCAS, M.I., R.C. NEWELL & B. VELIMIROV, 1981. Heterotrophic utilization of mucilage released during fragmentation of kelp (Ecklonia maxima and Laminaria pallida). II. Different utilization of dissolved organic components from kelp mucilage. Mar. Ecol. Prog. Ser. 4 : 43-55.

LUCAS, M.I. & R.C. NEWELL, 1984. Utilization of saltmarsh grass detritus by two estuarine bivalves: carbohydrase activity of crystalline style enzymes of the oyster Crassostrea virginica (Gmelin) and the mussel Geukensia demissa (Dillwyn). Mar. Biol. Letters 5 : 275-290.

LUCAS, M.I., S. PAINTING & D. MUIR, 1986. Estimates of carbon flow through bacterioplankton in the southern Benguela upwelling region, based on ^{3}H-Thymidine incorporation and predator-free incubations. Actes de colloque, IFREMER/CNRS. 3 : 375-383.

LUCAS, M.I., R.C. NEWELL, S.E. SHUMWAY, L.J. SEIDERER & R. BALLY, 1987. Particle clearance and yield in relation to bacterioplankton and suspended particulate availability in estuarine and open coast populations of the mussel Mytilus edulis. Mar. Ecol. Prog. Ser. 36 : 215-224.

LUND, J.W.G., C. KIPLING & E.D. LE CREN, 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11 : 143-170.

MANAHAN, D.T., 1983. The uptake and metabolism of dissolved amino acids by bivalve larvae. Biol. Bull, 164 : 236-250.

MANAHAN, D.T., S.H. WRIGHT & G.C. STEPHENS, 1982. Transport of dissolved amino acids by the mussel, Mytilus edulis: Demonstration of net uptake from natural sea water. Science 215 : 1253-1255.

MANAHAN, D.T., S.H. WRIGHT & G.C. STEPHENS, 1983. Simultaneous determination of net uptake of 16 amino acids by a marine bivalve. Am. J. Physiol. 244 (6) 832-838.

MANN, K.H., N. JARMAN & G. DIECKMANN, 1979. Development of a method for measuring the productivity of the kelp Ecklonia maxima. Trans. Roy. Soc. S. Afr., 44 (3) : 27-41.

McHENERY, J.G. & T.H. BIRKBECK, 1985. Uptake and processing of cultured microorganisms by bivalves. J. Exp. Mar. Biol. Ecol. 90 : 145-163.

McHENERY, J.G., T.H. BIRKBECK & J.A. ALLEN, 1979. The occurrence of lysozyme in marine bivalves. Comp. Biochem. Physiol. 63B : 25-28.

MILLER, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31(3) : 426-428.

MIRZA, M & M. SERBAN, 1981. Isolation and characterization of C_1 cellulases from Mya arenaria. Rev. Roum. Biochim. 18(1) : 33-38.

MONAR, I., 1972. Analysenautomat zur simultanen Mikrobestimming von C, H und N. Mikrochim. Acta. 784-806.

MONTEIRO, P.M.S., 1986. The potential for the use of high performance liquid chromatography in marine research. Trans. R. Soc S. Afr. 46(1) : 15-26.

MONTEIRO, P.M.S., E.J. BACON & M.J. ORREN, 1986. The South African SIBEX I cruise to Prydz Bay region, 1984: determination of photosynthetic pigments and their breakdown products by high performance liquid chromatography. S. Afr. J. Antarct. Res. 16(1) : 21-24.

MORRIS, I., 1971. An introduction to the algae. Hutchinson and Co. London. pp 189.

MORTON, B., 1983. Feeding and digestion in Bivalves. In: A.S.M. Salueddin and K.M. Wilbur (eds). The Mollusca, Vol 5, Physiology Part 2. pp 65-147. Academic press, New York.

MORTON, J.E., 1956. The tidal rythm and action of the digestive system of the lamellibranch Lasea rubra. J. mar. biol. Ass. UK. 35 : 563-586.

MUIR, D.G., 1986. Bacterial populations and their activity in the Benguela upwelling system. PhD Thesis. Department of Zoology, University of Cape Town, Rondebosch, South Africa.

MUIR, D.G., L.J. SEIDERER, C.L. DAVIS, S.J. PAINTING & F.T. ROBB, 1986. Filtration, lysis and absorption of bacteria by mussels Choromytilus meridionalis collected under upwelling and downwelling conditions. S. Afr. J. mar. Sci. 4 : 169-179.

MURAMATSU, T. & K. EGAWA, 1980. Isozymes of alginate lyase in the midgut gland of Turbo cornutus. Agric. Biol. Chem. 44 : 2587-2594.

MURAMATSU, T., S. HIROSE & M. KATAYOSE, 1977. Isolation and properties of alginate lyase from the mid-gut gland of wreath shell Turbo cornutus. Agric. Biol. Chem. 44 : 2587-2594.

NELSON, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153 : 375-380.

NEWELL, R.C., 1965. The role of detritus in the nutrition of two marine deposit feeders, the prosobranch Hydrobia ulva and the bivalve Macoma balthica. J. Zool. 144: 25-44.

NEWELL, R.C., 1981. The role of decomposers in the inshore region of the west coast. Trans. R. Soc. S. Afr. 44(3) : 327-334.

NEWELL, R.C., M.I. LUCAS, B. VELIMIROV & L.J. SEIDERER, 1980a. Quantitative significance of dissolved organic losses following fragmentaion of kelp (Ecklonia maxima and Laminaria pallida). Mar. Ecol. Prog. Ser. 2 : 45-59.

NEWELL, R.C., I. PARKER & P.A. COOK, 1980b. A possible role of -amylase isoenzymes from the style of the mussel Choromytilus meridionalis (Krauss) following thermal acclimation. J. Exp. Mar. Biol. Ecol. 47 : 1-8.

NEWELL, R.C., & M.I. LUCAS, 1981. The quantitative significance of dissolved and particulate organic matter released during fragmentation of kelp in coastal waters. Kieler. Meeresforsch. Sonderh. 5 : 356-369.

NEWELL, R.C., M.I. LUCAS & E.A.S. LINLEY, 1981. Rate of degradation and efficiency of conversion of phytoplankton debris by marine microorganisms. Mar. Ecol. Prog. Ser. 6 : 123-136.

NEWELL, R.C., J.G. FIELD & C.L. GRIFFITHS, 1982. Energy balance and significance of micro-organisms in a kelp bed community. Mar. Ecol. Prog. Ser. 8 : 103-113.

NEWELL, R.C. & J.G. FIELD, 1983. The contribution of bacteria and detritus to carbon and nitrogen flow in a benthic community. Mar. Biol. Letters 4 : 23-36.

NEWELL, R.I.E. & S.J. JORDAN, 1983. Preferential ingestion of organic material by the American oyster Crassostrea virginica. Mar. Ecol. Prog. Ser. 13 : 47-53.

OKADO, G., T. NISIZAWA & K. NISIZAWA, 1966. Cellulases of a marine mollusc, Dollabella sp. Biochem. J. 99 : 214-221.

OLIVIERI, E.T., 1983. Colonization, adaptations and temporal changes in diversity and biomass of a phytoplankton community in upwelled water of the Cape Peninsula, South Africa, in December 1979. S. Afr. J. mar. Sci. 1 : 77-109.

ONISHI, T., M. SUZUKI & R. KIKUCHI, 1985. The distribution of polysaccharide hydrolase activity in gastropods and bivalves. Bull. Jpn. Soc. Sci. Fish. 51(2) : 301-308.

PARSONS, T.R., M. TAKAHASHI & B. HARGRAVE, 1984. Biological Oceanographic Processes. Pergamon Press, Oxford. pp 330

PAYNE, D.W., N.A. THORPE & E.M. DONALDSON, 1972. Cellulolytic activity and the study of the bacterial population in the digestive tract of Scrobicularia plana (da Costa). Proc. Malacol. Soc. London 40 : 147-160.

PEQUIGNAT, E., 1973. A kinetic and autoradiographic study of the direct assimilation of amino acids and glucose by organs of the mussel Mytilus edulis. Mar. Biol. 19 : 227-244.

PERNAS, R.V., I.R. MARTINEZ & M.F. RAMA, 1981. Purifacation and properties of the two major forms of β-D-galactosidase from lysosymes of Mytilus edulis hepatopancreas. Comp. Biochem. Physiol. 69(B) : 851-857.

PIERSON, W.M., 1983. Utilization of eight algal species by the bay scallop Argopecten irradians concentricus (Say). J. Exp. Mar. Biol. Ecol. 68 : 1-11.

POLLOCK, D.E., 1978. Growth and production rates of the rock lobster Jasus lalandii (H. Milne-Edwards). PhD thesis, University of the Witwatersrand, South Africa.

PRIEUR, D., 1981. Experimental studies on trophic relationships between marine bacteria and bivalve molluscs. Kieler. Meeresforsch. Sonderh. 5 : 376-383.

PRIEUR, D., 1982. La microflore du tractus digestif des bivalves marins etude experimentale chez la moule, Mytilus edulis. Malocologia 22(1-2) : 653-658).

PRIM, P. & J.M. LAWRENCE, 1975. Utilization of marine plants and their constituents by bacteria isolated from the gut of echinoids (Echinodermata). Mar. Biol. 33 : 167-173.

PROBYN, T.A., 1985. Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system. Mar. Ecol. Prog. Ser. 22 : 249-258.

PROBYN, T.A. & C.D. McQUAID, 1985. In situ measurements of nitrogenous nutrient uptake by kelp Eklonia maxima and phytoplankton in a nitrate-rich upwelling environment. Mar. Biol. 88 : 149-154..

PURCHON, R.D., 1971. Digestion in filter feeding bivalves- a new concept. Proc. Malacol. Soc. London 39 : 563-586.

RAYMONT, J.E.G., 1980. Plankton and productivity in the oceans. Vol 1. Phytoplankton. Pergamon Press.

REID, F.G.B., 1966. Digestive tract enzymes in the bivalves Lima hians Gmelin and Mya arenaria L. Comp. Biochem Physiol. 17 : 417-433.

RICHARDS, F.A. & T.G. THOMPSON, 1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. J. Mar. Res. 11 : 156-172.

RIEMANN, B., 1978. Carotenoid interferences in the spectrophotometric determination of chlorophyll degradation products from natural populations of phytoplankton. Limnol. Oceanogr. 23 : 1059-1066.

RIEMANN, B., 1982. Measurement of chlorophyll a and its degradation products: a comparison of methods. Archiv. Fuer Hydrobiologie Beih. 16 :19-24.

RIVERS, D.B., S.J. GRACHECH, L.C. WOODFORD & G.H. EMERT, 1984. Limitations of the DNS assay for reducing sugars from saccharified ligno-cellulosics. Biotechnol. Bioeng. 26 : 800-802.

ROBYT, J.F. & W.J. WHELAN, 1972. Reducing value methods for maltodextrin: Chain length dependence of alkaline 3.5 dinitrosalicylate and chain length independence of alkaline copper. Anal. Biochem. 45 : 510-516.

ROSIOU, N. & C. IACOVACHE, 1980. Partial characterization of the purified α-amylase from the soft clam Mya arenaria L. Rev. Roum. Biochim. 17 (4) : 285-290.

SCHWARTZ, S.J. & J.H. VON ELBE, 1982. High performance liquid chromatography of plant pigments. J. Liq. Chromatography. 5(Suppl. 1) : 43-73.

SCOR-UNESCO: 1966. Monographs on oceanographic methodology. Vol 1 17 pp. Paris U.N.E.S.C.O..

SEIDERER, L.J. & R.C. NEWELL, 1979. Adjustment of the activity of α-amylase extracted from the style of the black mussel Choromytilus meridionalis (Krauss) in response to thermal acclimation. J. Exp. Mar. Biol. Ecol. 39 : 79-86.

SEIDERER, L.J., R.C. NEWELL & P.A. COOK, 1982. Quantitative significance of style enzymes from two marine mussels (Choromytilus meridionalis Krauss and Perna perna Linnaeus) in relation to diet. Mar. Biol. Letters 3 : 257-271.

SEIDERER, L.J., C.L. DAVIS, F.T. ROBB, & R.C. NEWELL, 1984. Utilization of bacteria as a nitrogen resource by kelp bed mussel Choromytilus meridionalis. Mar. Ecol. Prog. Ser. 15 : 109-116.

SEIDERER, L.J. & R.C. NEWELL, 1985, Relative significance of phytoplankton, bacteria and plant detritus as carbon and nitrogen resources for the kelp bed filter feeder Choromytilus meridionalis. Mar. Ecol. Prog. Ser. 22 : 127-139.

SEIDERER, L.J., R.C. NEWELL, K. SCHULTES, F.T. ROBB & C.M. TURLEY, 1987. Novel bacteriolytic activity associated with the style microflora of the mussel Mytilus edulis. J. Exp. Mar. Biol. Ecol. In press.

SHALLENBERGER, R.S., C. SEARLES & B.A. LEURS, 1974. Laminarinase activity in the crystalline style of the surf clam (Spissula solidissima). Experimentia 30 : 597-598.

SHEVCHENKO, N.M., T.N. ZVYAGINTSEVA & L.A. ELYAKOVA, 1986. Mode of action of endo-(1-3)-β-D-glucanases from marine molluscs on the laminarin from Laminaria cichorioides: the structure and the inhibitory effect of the resulting (1-3;1-6)-β-D-gluco-oligosaccharides. Carbohydr. Res. 152 : 261-273.

SHIMURA, S. & Y. FUJITA, 1975. Changes in the activity of fucoxanthin-excited photosynthesis in the marine diatom Phaeodactylum tricornutum grown under different culture conditions. Mar. Biol. 33 : 185-194.

SHIRAKI, M., M. YOSHIURA & K. IRIYAMA, 1978. Rapid and easy separation of chlorophylls, their derivatives, and plant yellow pigments by thin-layer chromatography. Chemistry letters. Chemical Society of Japan, Tokyo, Japan pp 103-104.

SHUMANN, F.R. & C.J. LORENZEN, 1975. Quantitative degradation of chlorophyll by a marine herbivore. Limnol. Oceanogr. 20 : 580-586.

SHUMWAY, S.E., T.L. CUCCI, R.C. NEWELL & C.M. YENTSCH, 1985. Particle selection, ingestion and absorption in filter-feeding bivalves. J. Exp. Mar. Biol. Ecol. 91 : 77-92.

SMUCKER, R.A. & D.A. WRIGHT, 1984. Chitinase activity in the crystalline style of the American oyster Crassostrea virginica. Comp. Biochem. Physiol. 77A : 239-241.

SOMOGYI, M., 1952. Notes on sugar determination. J. Biol. Chem. 195 : 19-23.

SOROKIN, Y.I., 1972. Bacteria as a food source for coral reef fauna. Oceanology 12: 169-177.

SOVA, V.V., L.L. ELYAKOVA & V.E. VASKOVSKY, 1970. The distribution of laminarinases in marine invertebrates. Comp. Biochem. Physiol. 32 : 459-464.

STARK, J.R. & R.S. WALKER, 1983. Carbohydrate digestion in Pecten maximis. Comp. Biochem. Physiol. 70B : 521-525.

STONE, B.A. & J.E. MORTON, 1958. The distribution of cellulases and related enzymes in Mollusca. Proc. Malac. Soc. Lond. 33 : 127-141.

STRAIN, H.H., J. SHERMA & M. GRANDOLFO, 1968. Comparative chromatography of the chloroplast pigments. Anal. Biochem. 24 : 54-69.

STRAIN, H.H., B.T. COPE & W.A. SVEC, 1971. Analytical procedures for the isolation, identification, estimation and investigation of the chlorophylls. In: A. San Pietro (ed.), Methods in Enzymology 23 : 452-476.

STRICKLAND, J.D.H. AND T.R. PARSONS, 1972. A practical handbook of sea water analysis, 2nd Ed. Bull. Fish. Res. Bd. Can. 167 : 310 pp.

STUART, V., 1982. Absorbed ration, respiratory costs and resultant scope for growth in the mussel Aulacomya ater (Molina) fed on a diet of kelp detritus of different ages. Mar. Biol. Letters 3 : 289-306.

STUART, V., M.I. LUCAS & R.C. NEWELL, 1981. Heterotrophic utilization of particulate matter from the kelp Laminaria pallida. Mar. Ecol. Prog. Ser. 4 : 337-348.

STUART, V., J.G. FIELD & R.C. NEWELL, 1982a. Evidence for absorption of kelp detritus by the ribbed mussel Aulacomya ater using a new ^{51}Cr-labelled microsphere technique. Mar. Ecol. Prog. Ser. 9 : 263-271.

STUART, V., R.C. NEWELL & M.I. LUCAS, 1982b. Conversion of kelp debris and faecal material from the mussel Aulacomya ater by marine microorganisms. Mar. Ecol. Prog. Ser. 1 : 47-57.

STUART, V. & D.W. KLUMPP, 1984. Evidence for food resource partitioning by kelp bed filter feeders. Mar. Ecol. Prog. Ser. 16 :27-37.

SUMNER, A.T., 1983. Some aspects of turnover in the digestive gland cells of Helix aspersa. J. Molluscan. Stud. 49(2) : 175

SUMNER, J.B., 1921. Dinitrosalicylic acid: a reagent for the estimation of sugar in normal and diabetic urine. J. Biol. Chem. 47 : 5-14.

SUMNER, J.B., 1924. The estimation of sugar in diabetic urine using dinitrosalicylic acid. J. Biol. Chem. 62 : 287-290.

SUMNER, J.B. & S.F. HOWELL, 1935. A method for determination of saccharase activity. J. Biol. Chem. 108 : 51-54.

TRAINER, D.G. & E.K. TILLINGHAST, 1982. Amylolytic activity of the crystalline style of Mya arenaria (Bivalvia, Mollusca). Comp. Biochem. Physiol. 72A : 99-103.

VELIMIROV, B., J.G. FIELD, C.L. GRIFFITHS & P. ZOUTENDYK, 1977. The ecology of kelp bed communities in the Benguela upwelling system. Estuar. Coast. Shelf Sci. 14 : 1-11.

VELIMIROV, B., 1980. Formation and potential trophic significance of marine foam near kelp beds in the Benguela upwelling system. Mar. Biol. 58 : 311-318.

WAINWRIGHT, P.F. & K.H. MANN, 1982. Effect of antimicrobial substances on the ability of the mysid shrimp Mysis stenolepsis to digest cellulose. Mar. Ecol. Prog. Ser. 7 : 309-313.

WATERBURY, J.B., C.B. CALLOWAY & R.D. TURNER, 1983. A cellulolytic nitrogen fixing bacterium cultured from the gland of Deshayes in shipworms (Bivalvia: Teredinidae). Science 221 : 1401-1403.

WHITNEY, D.E. & W.M. DARLEY, 1979. A method for the determination of chlorophyll a in samples containing degradation products. Limnol. Oceanogr. 24 : 183-186.

WIDDOWS, J., P. FIETH, & C.M. WORRAL, 1979. Relationships between seston, available food and feeding activity in the common mussel Mytilus edulis. Mar. Biol. 50 : 195-207.

WIDDOWS, J., P. DONKIN, P.N. SALKELD, J.J. CLEARY, D.M. LOWE, S.V. EVANS & P.E. THOMSON, 1984. Relative importance of environmental factors in determining physiological differences between two populations of mussels (Mytilus edulis). Mar. Ecol. Prog. Ser. 17 : 33-47.

WILBUR. K.M. 1983. (ed. in chief). The Mollusca. 6 volumes. Academic Press. New York.

WILDISH, D.J. & D.D. KRISTMANSON, 1984. Importance to mussels of the benthic boundary layer. Can. J. Fish. Aquat. Sci. 41 : 1618-1625.

WINTER, J.E., 1978. A review of the knowledge of suspension feeding in lamellibranchiate bivalves with special reference to artificial aquaculture systems. Aquaculture 13 : 1-33.

WOJTOWICZ, M.B., 1972. Carbohydrases of the digestive gland and the crystalline style of the Atlantic deep-sea scallop (Placopecten magellanicus, Gmelin). Comp. Biochem. Physiol. 43A : 131-141.

WRIGHT, R.T., R.B. COFFIN, C.P. ERSING & D. PEARSON, 1982. Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol. Oceanogr. 27(1) : 91-98.

WRIGHT, S.H. & G.C. STEPHENS, 1978. Removal of amino acids during a single passage of water across the gill of marine mussels. J. Exp. Zool. 205 : 337-352.

WRIGHT, S.W. & J.D. SHEARER, 1984. Rapid extraction and high performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton. J. Chromatogr. 294 : 281-295.

WULFF, F.V. & J.G. FIELD, 1983. Importance of different trophic pathways in a nearshore benthic community under upwelling and downwelling conditions. Mar. Ecol. Prog. Ser. 12 : 217-228.

YENTSCH, C.S. & D.W. MENZEL, 1963. A method for the determination of phytoplankton, chlorophyll and phaeophytin by fluorescence. Deep Sea Res. 10 : 221-231.

YOKOE, Y. & I. YASUMASU, 1964. The distribution of cellulases in invertebrates. Comp. Biochem. Physiol. 13 : 323-338.

YONGE, C.M., 1923. Studies on the comparative physiology of digestion. Brit. J. Exp. Biol. 1 : 15-63.

APPENDIX A

REAGENTS

Agar plates

1,5% agar in sea water growth medium

Sea water growth medium:

3 parts 0,45 µm filtered sea water

1 part glass distilled water

0,5% w/v peptone

0,1% w/v yeast extract

0,01% ferric phosphate

Dinitrosalicylic acid reagent

2 g of 3,5-dinitrosalicylic acid were dissolved in 40 ml 2N NaOH. A further 100 ml of distilled water were added together with 60 g of sodium potassium tartrate. The solution was heated and stirred to dissolve the reagents and made up to 200 ml with distilled water.

Folin-Lowry Protein Determination

Reagents

A 1 N NaOH

B Sodium potassium tartrate - $CuSO_4$-Na_2CO_3 mixture was made up fresh daily as follows:

0,5 ml 2% w/v sodium potassium tartrate

0,5 ml 1% w/v $CuSO_4$

5,0 ml 20% w/v Na_2CO_3

This was made up to 50 ml with distilled water

C Folin reagent: 1 M 2N Folin Ciocalteus (Merck) reagent was diluted to 2,5 ml with distilled water.

Calibration

A stock solution of crystalline bovine serum albumin was diluted to obtain standards with a final protein content of 40, 80, 200, 300, 400, 500 and 600 $\mu g.ml^{-1}$, which were used to construct a calibration curve.

Procedure

To 250 µl of sample (standard) were added 250 µl A and 2,5 ml B. This was vortexed and allowed to stand for 10 minutes. To this was added 250 µl C and after standing for 30 minutes the solution was read against H_2O at 660 nm. Blank values were subtracted from readings.

Nelson-Somogyi Reagents

Somogyi reagent A:

24 g Na_2CO_3

16 g $NaHCO_3$

12 g Sodium potassium tartrate

144 g Na_2SO_4

These were dissolved in 800 ml distilled water, boiled, and sealed in a brown bottle.

Somogyi reagent B:

4 g $CuSO_4.5H_2O$

36 g Na_2SO_4

These were dissolved in 200 ml distilled water and stored in a brown bottle. Somogyi reagents A and B were mixed in a ratio of 4:1 on the day of use.

Nelson reagent:

25 g $(NH_4)_6MO_7O_{24}.4H_2O$ were dissolved in 450 ml distilled water. 21 ml conc. H_2SO_4 and 3 g Na_2HAsO_4 dissolved in 25 ml distilled water was added. The solution was kept at 37°C for 24 hours and stored in a brown bottle.

Phosphate buffer pH 6,9 with 150 mN NaCl

A 2,722 g KH_2PO_4 was dissolved in 1 l distilled water.

B 3,560 g $NaHPO_4$ was dissolved in 1 l distilled water.

389 ml of A and 611 ml of B were mixed and 8,766 g NaCl was added.

APPENDIX B

CHEMICALS

Acetone - Merck.

Alginic acid - Sigma.

Ammonium acetate - Protea Chemicals.

Ampicillin - Boehringer Mannheim.

-amylase from Bacillus subtilus - Sigma, crude type III.

Arabinose - Merck.

Biocide 'D' - Biocide Products, Woodstock, Cape Town.

Bovine serum albumin, gel filtration standard - Boehringer Mannheim.

Bovine serum albumin, Lowry protein standard - BDH, England.

Carboxymethyl cellulose - BDH, England.

Chlorophyll a - Sigma.

Cellobiose - Merck.

Cellulase from Aspergillus niger - Sigma, practical grade II.

Cytochrome C - Boehringer Mannheim.

Dextrin - Merck.

Fructose - Merck.

Galactose - Merck.

Glucose - Merck.

Lactose - Merck.

Laminarin - Sigma.

Laminarinase - Sigma.

Lysozyme - Boehringer Mannheim.

Maltose - Merck.

Mannose - Merck.

Methanol - Merck.

Ovalbumin - Boehringer Mannheim.

Oyster glycogen - BDH, England.

Ribose - Merck.

Sephacryl A-200 - Pharmacia Fine Chemicals.

Starch - BDH, England.

Streptomycin sulphate - Boehringer Mannheim.

Tetrabutyl ammonium acetate - Protea Chemicals.

Yeast extract - Difco Laboratories, Detroit, Michigan.

APPENDIX C

SUPPORTING PAPERS

Comp. Biochem. Physiol. Vol. 67A, pp. 691 to 694

0300-9629/80/1201-0691$02.00/0

REGULATION OF HAEMOLYMPH OSMOLARITY AND IONS IN THE GREEN PROTEA BEETLE, *TRICHOSTETHA FASCICULARIS*, DURING DEHYDRATION AND REHYDRATION

P. J. FIELDING and SUSAN W. NICOLSON
Department of Zoology, University of Cape Town, Rondebosch 7700, South Africa

(*Received* 7 *March* 1980)

Abstract—1. During dehydration protea beetles lose weight rapidly, but their haemolymph osmolarity and sodium and potassium concentrations are well regulated.
2. Drinking restores the haemolymph volume and osmolarity to the original levels, but not the cation concentrations.
3. Osmoregulation in the protea beetle is compared with that in other insects.
4. The diet and behaviour of the beetle are important factors in its water balance.

INTRODUCTION

One of the most conspicuous insects in *Protea* flowerheads is the green protea beetle, *Trichostetha fascicularis* Linn (Scarabaeidae: Cetoniinae). This large and brightly coloured beetle is endemic to South Africa and always closely associated with the genus *Protea* (Gess, 1968). Its life history has been briefly described by Skaife (1955): for eighteen months the larvae live on organic matter in the soil beneath termite mounds, then the adult beetles emerge in autumn to feed on *Protea* nectar and pollen.

Because of its partially liquid diet, the protea beetle may not need to conserve water as efficiently as other terrestrial insects. Studies of the effect of dehydration on xeric and desert species have demonstrated their remarkable ability to maintain water and ion balance under adverse conditions (Edney, 1977). Water loss in these insects is kept to a minimum, and the internal environment is regulated within rather narrow limits. The present investigation was designed to show whether the protea beetle is indeed more vulnerable to water loss by transpiration, and to what extent it can control its haemolymph composition.

METHODS

Beetles were collected from *Protea grandiceps* growing at Kirstenbosch Botanic Gardens, Cape Town. For the dehydration experiments a desiccator containing silica gel provided a relative humidity of 10–15%. The beetles were not confined in individual vials, but were allowed to roam freely about the desiccator. After 6 days of dehydration they were allowed access to cotton wool soaked with distilled water, and were maintained at 50–60% r.h. for a further 3 days. All experiments were carried out at 26 ± 1°C.

Ten of the beetles were marked with queen bee numbers (Graze, Weinstadt, West Germany) and weighed at 1000 hr each day, to the nearest 0.1 mg, during the 9 day experimental period. The remaining beetles were killed at appropriate intervals for measurements of haemolymph volume, osmolarity and cation concentrations during dehydration and rehydration.

Haemolymph volume was estimated by the gravimetric method of Richardson *et al.* (1931), which consists of dissecting a weighed insect and carefully removing the haemolymph with absorbent tissue before reweighing. Since the specific gravity of insect haemolymph is approximately unity (Altman & Dittmer, 1974), the weight in mg was taken as equal to the volume in μl.

Samples of haemolymph for analysis were obtained by decapitating the insect and collecting the haemolymph in micropipettes (Drummond Scientific Co.). The few samples contaminated with yellow gut fluid were discarded. Osmolarity of the haemolymph was determined immediately after collection, on a Wescor 5100B Vapour Pressure Osmometer. Diluted haemolymph samples were analysed for sodium and potassium on an IL 243 Flame Photometer (Instrumentation Laboratory).

RESULTS

During dehydration the marked beetles lost weight at the rate of 4.3% of their original weight per day (Fig. 1). On the first day their weight loss was increased by the excretion of a brown fluid. After 6 days of dehydration body weight was 74.3 ± 1.0% (mean ± SE) of original weight. Recovery was good if the beetles were given water at this stage, their weight returning to 89.7 ± 1.4% of original weight during 24 hr of rehydration. Dehydration for longer than 6 days, however, resulted in a high mortality.

Figure 2 shows the changes in haemolymph volume during dehydration and rehydration. Because of the variation in size between individuals, the data are corrected to a standard animal weighing initially 1.407 g. Desiccation caused the haemolymph volume to fall sharply, from 239 ± 18 μl in control insects to 65 ± 3 μl on day 6. Drinking, however, returned the haemolymph volume to normal. During the 3 days of rehydration the haemolymph volume did not differ significantly from that of the controls ($P > 0.05$).

The increases in haemolymph osmolarity and ion concentrations resulting from desiccation were less than would be expected from the volume changes. Figure 3 shows a 63% increase in osmolarity during the 6 days of desiccation, followed by the restoration

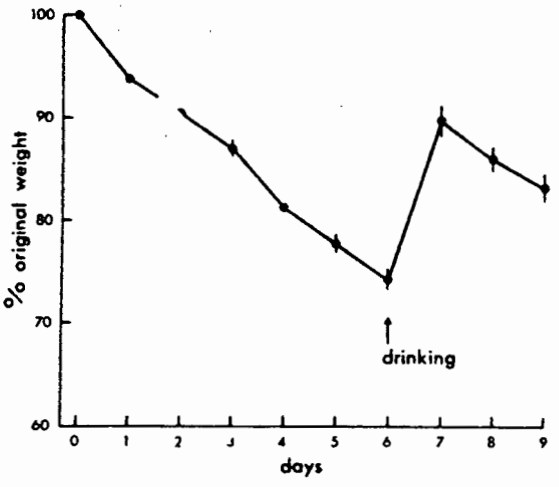


Fig. 1. Weight changes of protea beetles during dehydration and rehydration. Time of drinking (day 6) shown by arrow. Vertical lines indicate ± 1 SE, except where symbol exceeds size of the SE. $N = 10$.

of normal values after drinking. Differences in haemolymph osmolarity of control and rehydrated beetles were not significant ($P > 0.05$).

Figure 4 shows the effects of dehydration and rehydration on sodium and potassium concentrations in the haemolymph. Sodium levels increased by only 31% during 6 days of dehydration, from 107 ± 8 mM to 140 ± 13 mM. The potassium concentration was stable for the first 4 days, then rose steeply. By 24 hr after drinking, the concentrations of both ions had dropped significantly below normal values ($P < 0.05$). Even by the third day of rehydration the normal concentrations had not been restored.

DISCUSSION

It is difficult to compare osmotic and ionic regulation in different insects unless the severity of dehydration during experiments is taken into account. The insects may be deprived of both food and water and subjected to extremely low humidities, or instead

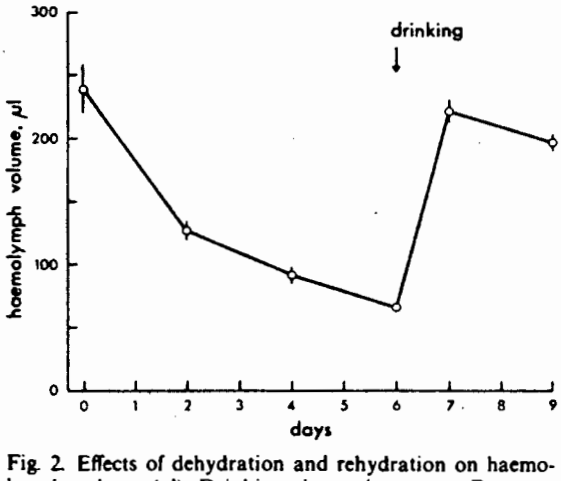


Fig. 2. Effects of dehydration and rehydration on haemolymph volume (µl). Drinking shown by arrow. Data are corrected to a standard insect of initial weight 1.407 g. Vertical lines indicate ± 1 SE, unless symbol larger than SE. $N = 6$.

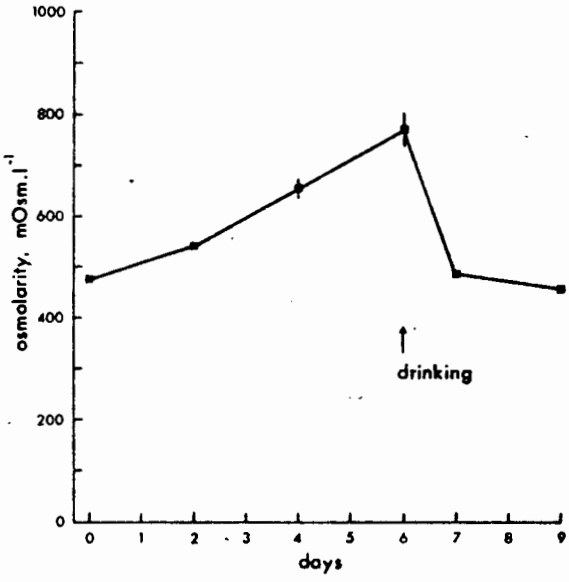


Fig. 3. Changes in haemolymph osmolarity (mOsm/l) during 6 days of dehydration, followed by 3 days of rehydration. Time of drinking shown by arrow. SE's given by vertical lines, unless smaller than symbols, and $N = 6$.

drinking water alone may be withheld and the humidity not controlled. In the latter case, although provided with food, cockroaches may be reluctant to eat (Tucker, 1977). Because of this variation in experimental conditions, the osmoregulatory ability of an insect is best assessed by relating changes in its haemolymph volume to the corresponding changes in haemolymph composition.

The haemolymph volume of the protea beetle is drastically reduced during desiccation and it seems probable that water is withdrawn from its haemo-

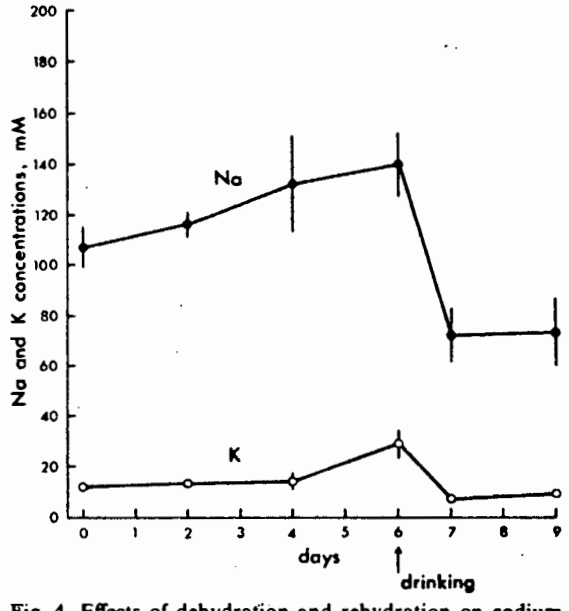


Fig. 4. Effects of dehydration and rehydration on sodium (●——●) and potassium (○——○) concentrations (mM) in the haemolymph of protea beetles. Time of drinking marked by arrow. Vertical lines indicate ± 1 SE, unless symbol larger than SE. $N = 6$.

lymph to maintain tissue water levels, as in other insects (Edney, 1977). A fall in average haemolymph volume from 239 μl to 65 μl during 6 days of dehydration led to an increase in osmolarity from 474 mOsm/l to 772 mOsm/l. In the absence of regulation the osmolarity would be 474 × 239/65 = 1742 mOsm/l. A similar calculation for the first day of rehydration, when the haemolymph volume increased from 65 μl to 221 μl, gives an expected osmolarity of 772 × 65/221 = 227 mOsm/l, whereas the measured value was 486 mOsm/l. Thus the protea beetle regulates its haemolymph osmolarity well during both dehydration and rehydration.

Not surprisingly, sodium and potassium ions exhibit a pattern of regulation similar to that for the total osmolarity. Sodium is the major haemolymph cation in many insects, particularly in those most familiar to physiologists, and in cockroaches the reversible storage of sodium ions in tissues such as the fat body has been clearly demonstrated as a mechanism of osmoregulation (Tucker, 1977; Hyatt & Marshall, 1977). Although both sodium and potassium are removed from the haemolymph of *Trichostetha* during dehydration, it is possible that the potassium and about 33% of the sodium are excreted rather than stored, since even after 3 days of rehydration the concentrations of these ions remain abnormally low (Fig. 4). Under these conditions other constituents of the haemolymph must be making an increased contribution to the total osmolarity, which does return to normal. Hyatt & Marshall (1978) have established that the cricket *Teleogryllus*, which is a relatively poor osmoregulator, controls its haemolymph composition by elimination of cations rather than their storage.

With the exception of the work on *Teleogryllus* just mentioned, most studies of this nature have concerned insects adapted to dry or desert environments. Table 1 compares osmoregulation in the protea beetle and three other insects: the desert tenebrionid *Onymacris*, the cockroach *Periplaneta* and the cricket *Teleogryllus*. By using the data from protea beetles desiccated for 4 days, it has been possible to compare the effects of a 62–66% drop in haemolymph volume in all four insects. The table shows that, although excellent in *Onymacris* and *Periplaneta*, osmoregulation is also quite good in the two species from more mesic environments, provided the period of desiccation is not prolonged.

High rates of evaporative water loss in desiccated protea beetles may include a large respiratory component. Protea beetles raise their thoracic temperatures well above ambient before initiating flight (Nicolson & Louw, 1980), and preflight warm-up was observed in some of the beetles confined in the desiccator. Like flight itself, this activity would result in elevated rates of oxygen consumption and therefore greater respiratory water losses.

Protea beetles may not actually experience problems of water shortage in their natural environment. Not all insects are forced to conserve water: in fact those feeding on blood or plant sap require special mechanisms for the rapid elimination of water (Edney, 1977). *Trichostetha*, feeding on *Protea* nectar, may have temporary problems of water excess, especially when the nectar is diluted by rain collecting in the flower-head. However, pollen is also important in the diet of protea beetles (Brunhuber, 1964; Nicolson and Louw, unpublished). The behaviour of the beetles serves to minimise water loss, in that they spend only a small proportion of their time in flight and are usually to be found foraging in *Protea* flowerheads or resting deep inside them. Depending on the shape and structure of the flower-head, the microclimate within it may differ considerably from conditions outside. The temperature inside the flowerhead of *P. grandiceps* can remain consistently higher than ambient temperature throughout the day (Nicolson & Louw, 1980), and nectar and rainfall may raise the humidity. Thus the protea beetle is very susceptible to water loss, but its diet and behaviour are significant factors in maintaining total water balance.

Acknowledgements—We wish to thank Professor G. N. Louw and Dr J. P. Loveridge for advice, and Professor H. B. Rycroft for permission to collect beetles from Kirstenbosch Botanic Gardens. Thanks are also due to the South African Council for Scientific and Industrial Research for financial assistance.

Table 1. Osmoregulation in 4 different species of insect, all dehydrated sufficiently to cause a decrease in the haemolymph volume of approx 66%

	Onymacris	*Periplaneta*	*Trichostetha*	*Teleogryllus*
T°C	26	25	26	25
R.h., %	10–15	60	10–15	60
Fed/unfed	Not fed	Fed	Not fed	Fed
Initial wt (mg)	864	825	1407	624
Wt loss, %/day	0.9	3.4	4.3	9.3
Days	12	8	4	3
% Change in haemolymph volume	−66	−64	−62	−65
% Change in osmolarity	+14	+11	+38	+46
% Change in Na^+	+5	+14	+23	+94
% Change in K^+	+74	+22	+17	+102
$Na^+ + K^+$ as % total cations	62	90	46	77
Reference	Nicolson, 1980	Hyatt & Marshall, 1977	Present paper	Hyatt & Marshall, 1978

REFERENCES

ALTMAN P. L. & DITTMER D. S. (Eds) (1974) *Biology Data Book*, 2nd edn, Vol. III, pp. 1911–1912. Federation of American Societies for Experimental Biology, Bethesda.

BRUNHUBER B. S. (1964) An investigation into the mechanisms of orientation connected with feeding in the protea beetle *Trichostetha fascicularis* (Linn.) Unpublished Zoology Honours Project, University of Cape Town.

EDNEY E. B. (1977) *Water Balance in Land Arthropods*. Springer-Verlag, Berlin.

GESS F. W. (1968) Insects found on Proteas. *J. bot. Soc. S. Afr.* **54**, 29–33.

HYATT A. D. & MARSHALL A. T. (1977) Sequestration of haemolymph sodium and potassium by fat body in the water-stressed cockroach *Periplaneta americana*. *J. Insect Physiol.* **23**, 1437–1441.

HYATT A. D. & MARSHALL A. T. (1978) Regulation of haemolymph sodium and potassium during dehydration in the cricket *Teleogryllus oceanicus*. *Physiol. Entomol.* **3**, 207–211.

NICOLSON S. W. (1980) Water balance and osmoregulation in *Onymacris plana*, a tenebrionid beetle from the Namib Desert. *J. Insect Physiol.* **26**, 315–320.

NICOLSON S. W. & LOUW G. N. (1980) Preflight thermogenesis, conductance and thermoregulation in the protea beetle, *Trichostetha fascicularis* (Scarabaeidae: Cetoniinae). *S. Afr. J. Sci.* **76**, 124–126.

RICHARDSON C. H., BURDETTE R. C. & EAGLESON C. W. (1931) The determination of the blood volume of insect larvae. *Ann. ent. Soc. Am.* **24**, 503–507.

SKAIFE S. H. (1955) *Dwellers in Darkness: an Introduction to the Study of Termites*, pp. 84–86. Longmans, London.

TUCKER L. E. (1977) Effect of dehydration and rehydration on the water content and Na^+ and K^+ balance in adult male *Periplaneta americana*. *J. exp. Biol.* **71**, 49–66.

Benthic diatom biomass, production, and sediment chlorophyll in Langebaan lagoon, South Africa

P.J. Fielding[a], K.St.J. Damstra[b] and G.M. Branch

Department of Zoology, University of Cape Town, South Africa
Present address:[a] Department of Biology, University of Natal, King George V Av., Durban, South Africa.
[b]Department of Biological Sciences, University of Zimbabwe, Harare, Zimbabwe

Keywords : diatoms, biomass, chlorophylls, carbon fixation, sediments, lagoons, South Africa coast.

ABSTRACT

Direct measurements were made of the biomass of benthic diatoms in Langebaan lagoon, a sheltered lagoon on the west coast of South Africa. Highest biomasses occurred in the most sheltered areas and reached 18.9 $g.m^{-2}$ in the top 30 cm of sediment. A large biomass was found even at depths of 10-20 cm and 20-30 cm, often exceeding that in the 0-10 cm depth range. This contrasts with chlorophyll estimates of biomass, in which chlorophyll *a* declines rapidly below the photic zone. Very high sediment turnover rates by benthic macrofauna accounts for the presence of microalgae at depths of 30 cm below the sediment surface. Currents or waves (and hence particle size) are inversely correlated with diatom biomass. Chlorophyll *a* concentrations in the surface layers of the sediment may reach 322 µg Chl *a*.g^{-1} sediment, but generally values range from 0 to 34 µg Chl *a*.g^{-1} sediment. Like diatom biomass, chlorophyll *a* increases with declining waves and currents. Highest chlorophyll *a* concentrations occur in the top millimetre of sediment, declining to 35% of surface concentrations 5 cm below the surface. Rates of carbon fixation by benthic microalgae in the top 5mm of sediment were 17.38 mg $C.m^{-2}.h^{-1}$ at an exposed sandy beach, and 69.54 mg $C.m^{-2}.h^{-1}$ at a sheltered sand/mud beach. Benthic microfloral production rivals phytoplankton production and ranges from estimates of 63 g $C.m^{-2}.y^{-1}$ (sand) to 253 g $C.m^{-2}.y^{-1}$ (sand/mud). In the lagoon ecosystem,

production by benthic microalgae amounts to 22% of the total primary production of carbon.

INTRODUCTION

Diatoms frequently form important components of the benthos of sheltered, shallow marine environments. In such systems the benthic microalgal community may contribute considerably to overall primary productivity, and may also affect phytoplankton production by recycling nutrients within the benthos. In some cases the benthic primary productivity may rival that of the phytoplankton in the overlying waters (Cadee, 1980; Colijn , 1982; Varela & Penas, 1985 Lukatelich & McComb, 1986).

Primary production by littoral diatoms differs from that of open waters because all carbon fixation must take place in the top few millimetres of the sediment, since light penetration of the sediment is negligible (Pamatat, 1968; Gargas, 1970; Fenchel & Straarup, 1971; Cadee & Hegeman, 1974). However, microalgae (usually recorded as chlorophyll) are frequently found below this depth and are capable of photosynthesis when exposed to light (Steele & Baird, 1968; Pamatat, 1968; Hunding, 1971; Riznyk & Phinney, 1972; Cadee & Hegeman, 1974).

Vertical migration of diatoms regularly occurs over short distances of a few millimetres and may be tidally induced or diurnal (Aleem, 1950; Pomeroy, 1959; Palmer & Round, 1967; Colijn & van Buurt, 1975; Admiraal et al., 1982). However,

diatoms are frequently found so deep in the sediment that their limited mobility would be insufficient to allow them regular access to the surface and to adequate light for photosynthetic activity. For example, diatoms have been found at depths of 10 cm (Cadee, 1980;), 20 cm (Steele & Baird, 1968) and 30 cm (Branch & Pringle, 1987) in the sediment, although generally it is assumed that most benthic microalgae occur in the top few centimetres of the sediment.

Recently, measurements of chlorophyll concentrations and production have been made in several calm-water localities (Davis & McIntire,1983; Colijn & de Jonge, 1984; Nowicki & Nixon, 1985; Varela & Penas, 1985; Lukatelich & McComb, 1986). However, apart from the works of Fenchel and Straarup (1971), Admiraal et al. (1982) and de Jonge (1985) few attempts have been made to obtain a direct measure of benthic microalgal numbers, and biomass in the sediment is generally recorded as chlorophyll content. The chlorophyll a content of diatoms kept in the dark appears to decrease (Gargas & Gargas, 1982), which implies that estimates of biomass measured as chlorophyll content in the deeper sediment layers may be in error.

Comparatively little work has been done on the standing stocks and productivity of benthic microalgae in Southern African lagoons and estuaries, although Dye (1978) has measured their production in the Swartkops estuary. The present observations on benthic microalgae were made in

Langebaan lagoon, a partially enclosed marine system situated between 33°00' to 33°13'S and 17°57' to 18°08'E, on the south west coast of South Africa. The upper reaches of the lagoon are very sheltered and consist of shallow water saltmarshes and extensive sand flats, which support a large and varied diatom population (Giffen, 1975; Giffen, 1976). Nearer the mouth of the lagoon and in adjacent Saldanha Bay, strong tidal currents and wave action disturb the sediments so that there is a gradient of energy from the head of the lagoon to the open bay (Flemming, 1977 a,b).

Extensive research has been carried out on many aspects of the lagoon ecology (Christie and Moldan, 1977; Flemming, 1977 a,b; Henry et al., 1977; Puttick, 1977; Christie, 1981), but so far the contribution of the microalgal benthic primary producers to the whole system has not been determined. The following work was carried out to determine the distribution and biomass of benthic diatoms in the lagoon, and to relate this to the energy gradient that exists along the length of the lagoon, and to the chlorophyll a content of the sediment. Benthic microalgal biomass, determined by direct measurement, is compared with that of other benthic components and, on the basis of a short term measurement of productivity, the benthic production relative to that of macrophytes and phytoplankton is estimated.

MATERIALS AND METHODS

Diatom numbers and biomass.

Sediment samples were collected at ten stations around the lagoon (Figure 1) from the high, mid and low shore, and subtidally at about 30 cm below low water spring tides. Five cores, 30 cm long and 5 cm in diameter, were removed from the sediment at each tidal level, excluding the sites at Long Beach and Kraalbaai where subsurface rocks prevented collection of the deeper samples . Each core was sectioned into three depth intervals: 0-10 cm, 10-20 cm and 20-30 cm. All five replicates from each depth were mixed together and a 50 cm^3 subsample taken. Each subsample was processed with a modified Oostenbrink apparatus (Fricke, 1979) to extract the diatoms. Samples were stained with rose-bengal and numbers of live diatoms counted using a microscope. The dimensions of diatoms were measured using a graduated microscope eyepiece. Diatom volumes were then calculated and dry biomass estimated assuming a specific gravity of one and a water content of 80%.

Chlorophyll analyses

Sediments at seven of the ten stations were sampled for chlorophyll, i.e. Lynch Point, Long Beach, Klein Oesterwal, Bottelary, Geelbek, Churchhaven and Kraalbaai (Figure 1). Samples were taken between 0830 and 1230 hours at the high, mid and low shore. At each tidal level cores were used to

FIGURE 1

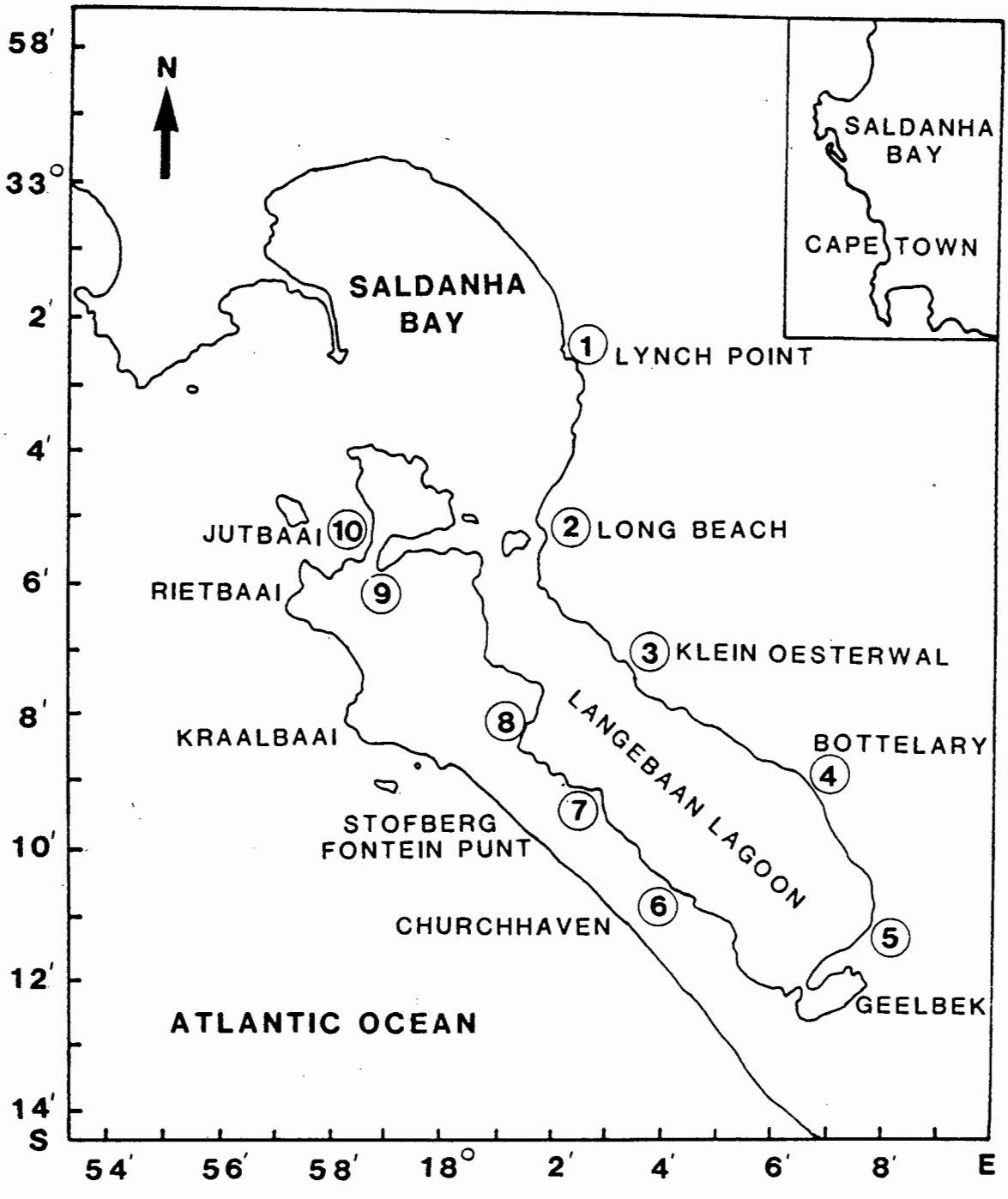


Figure 1. Saldanha Bay and Langebaan lagoon. Numbers indicate sampling stations around the edge of the lagoon.

obtain duplicate samples of 2-7 g at the surface and at 5, 15 and 25 cm below the surface of the sediment. Sediment samples were placed in stainless steel tubes containing 2 ml 90% acetone and a few milligrams of finely powdered $MgCO_3$ to prevent acidification (Strickland & Parsons, 1972).

To extract the chlorophyll, sediment samples were ground in the steel tubes, and the supernatant was removed. Functional chlorophyl a and phaeopigments were measured following the method of Lorenzen (1967). Chlorophyll b was also measured (Strickland & Parsons, 1972) to determine whether blue-green and euglenoid algae formed a component of the benthos. Chlorophyll a was expressed as µg Chlorophyll a.g^{-1} dry sediment.

Carbon fixation and Chlorophyll a

Four stations were chosen for simultaneous investigation of chlorophyll a levels and of rates of in situ ^{14}C fixation by benthic microalgae i.e. Long Beach, Klein Oesterwal, Bottelary and Geelbeck (Figure 1). Two light and one dark ^{14}C incubation experiments were conducted at mid and low tide levels at each of the four stations between 1200 and 1400 hours on successive sunny, cloudless days.

Incubation chambers (12 cm long by 5 cm diameter) were pressed into the sediment to a depth of 6 cm and a core removed. The bottoms of the cylinders were then stoppered. The enclosed cores were replaced in the holes from which they

had been removed. Exactly 100 ml of 0.45 µm filtered seawater was added to each cylinder and 1 ml of $NaH^{14}CO_3$ label (made up to 10 $\mu Ci.ml^{-1}$ in 0.45 µm filtered sea water and raised to pH 9.1) was added to each cylinder. After two hours the water covering each core was removed with a syringe. The cores were then removed intact from the cylinders and the top 5 mm of sediment was shaved off, lyophilized, and duplicate 20 to 30 mg subsamples were counted in Instagel (Marshall et al., 1973). Counts were corrected for quench using an internal standard and ^{14}C hexadecane . Carbon fixation was determined after Marshall et al. (1973), assuming a carbonate content of 25 mg $CO_3.l^{-1}$ for sea water (Strickland & Parsons, 1972). Carbon fixation was calculated per m^{-2} for the top 5 mm of sediment using weight:volume conversions for the sediment.

Adjacent to each incubation site duplicate samples of the top millimetre and the 2-5 millimetre depth interval were taken for chlorophyll analyses.

RESULTS AND DISCUSSION

Diatom biomass

The dry biomass of the benthic diatoms is shown in Figure 2. With the exception of one site (Stofbergfontein Punt), diatom biomasses in the southern lagoon were highest (18.97 $g.m^{-2}$ maximum) at subtidal and low tide sites, mid and high tide biomass values being considerably lower (0.07 to 9.99 $g.m^{-2}$).

FIGURE 2

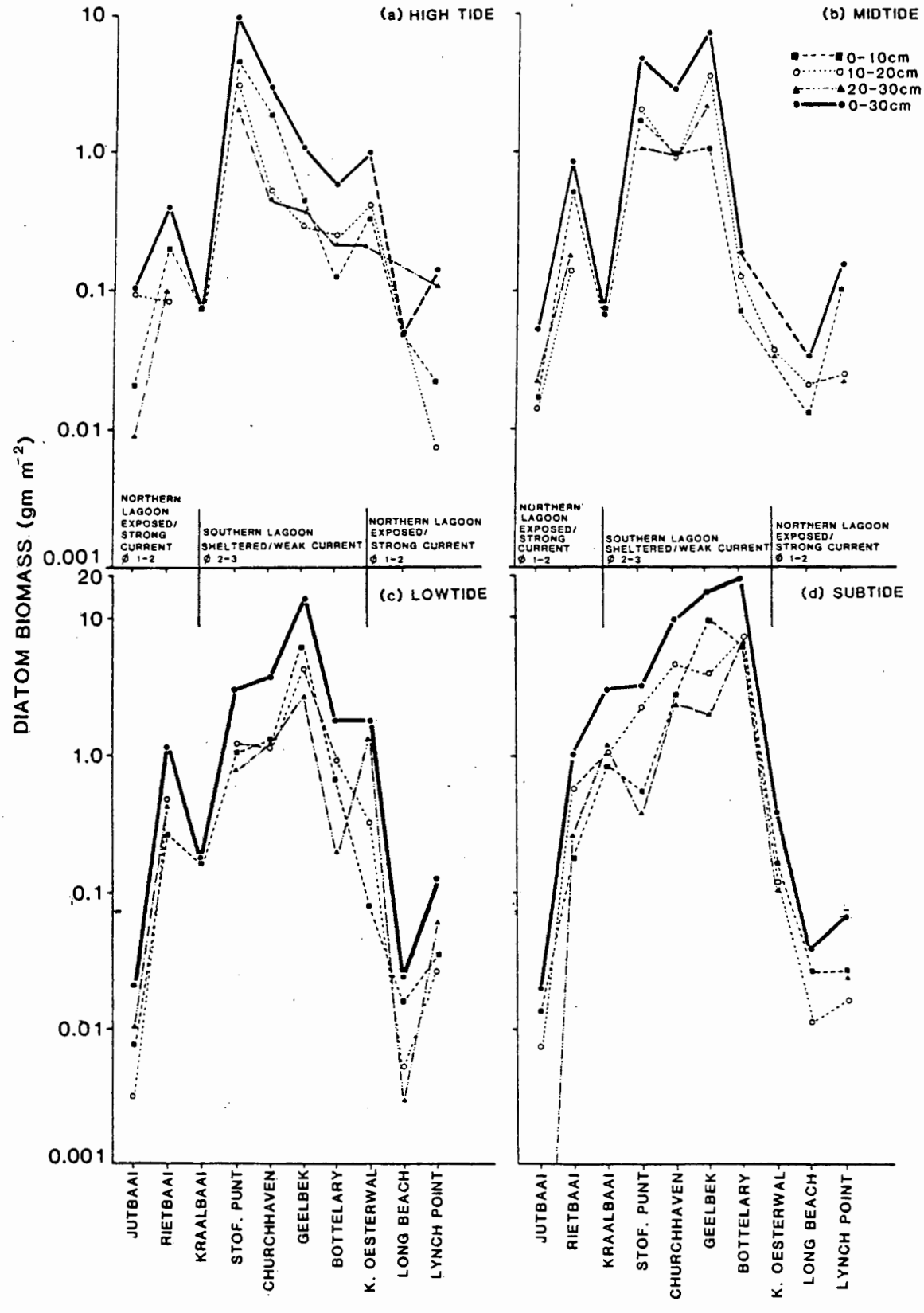


Figure 2. Diatom biomass at depths of 0-10 cm, 10-20 cm, 20-30 cm and 0-30 cm in the sediment at different tidal levels at stations around Langebaan lagoon. Degree of exposure, current strength and sediment phi values are also indicated.

Diatom biomass thus showed a very distinct North-South gradient similar to that of the mean sediment particle size and opposite to that of the energy of the currents (see Flemming, 1977a,b). Mean phi values are 2.5 to 3 in the southern and eastern lagoon and 1 to 2 in the western and northern lagoon. Benthic microalgal biomass, generally measured as chlorophyll a has previously been shown to be related to sediment type, finer sediments having higher chlorophyll a concentrations than coarser sediments (Riznyk & Phinney, 1972; Colijn & Dijkema, 1981; Davis & McIntire, 1983; Shaffer & Onuf, 1983). The degree of physical disturbance has also been found to affect biomass (Moss & Round, 1967; Hickman & Round, 1970; van den Hoek et al, 1979; Colijn & Dijkema, 1981), but in this case it is difficult to separate the effects of particle size and physical disturbance, since the currents which are the main physical peturbators, are also the main sediment particle sorting mechanism in the lagoon (Flemming, 1977 a,b). de Jonge (1985) has stressed the importance of mud particles as a substratum for diatoms and this may also partly account for the increasing diatom biomass towards the head of the lagoon. The mud content of the lagoon sediments is generally less than 1% but does increase very slowly towards the southern end of the lagoon, reaching 10% at Geelbek (Flemming, 1977b).

The effect of tidal elevation on biomass is not clear. Riznyk and Phinney (1972) found that the lower intertidal zone had approximately 1.5 times as many diatom cells as the mid intertidal zone, and twice as many as the upper intertidal

zone, while Colijn and de Jonge (1984) found the highest production at the highest tidal levels and Davis and McIntire (1983) found no effect of tidal height on algal biomass measured as chlorophyll a. Subtidal and low tide diatom biomasses at Langebaan were 1.3 to 43 times greater than the corresponding high tide biomasses at the southern end of the lagoon. This is possibly related to the greater physical stress high on the shore and the shorter periods of exposure to water-borne nutrients. However , this trend was reversed at sites exposed to wave action (e.g. Lynch Point, Long Beach and Jutbaai) where the mid and high tide levels had higher biomasses than low and subtidal sites. The differences between shore levels were however, greatly overshadowed by the far greater differences between stations, which spanned three orders of magnitude.

The benthic diatom density in Langebaan lagoon can be compared with that of the Ems-Dollard estuary in the northern hemisphere, using weight:volume conversions for Langebaan sediments and assuming that the diatoms are approximately evenly distributed throughout a depth of 30 cm of sediment (Figure 2). Admiraal et al. (1982) and de Jonge (1985) estimated cell densities of between $2x10^{8}.m^{-2}$ and $7x10^{10}.m^{-2}$ in the top 5 mm of the Ems-Dollard sediments. Langebaan lagoon diatom numbers in the top 5 mm of sediment ranged from $1.16x10^{4}$ cells.m^{-2} in the coarse sediments at Jutbaai to $6.3x10^{6}$ cells.m^{-2} in the finer sand of Bottelary. Thus diatom densities would appear to be somewhat lower at Langebaan.

It is also evident from Figure 2 that the greatest diatom biomass was not always found in the top layer of sediment. A large biomass was frequently found 10-20 cm or 20-30 cm below the surface of the substratum and, in many cases, biomass in these depth ranges exceeded that between 0 and 10 cm. Although viable diatoms have been found at considerable depths before (Fenchel & Straarup, 1971; Riznyk & Phinney, 1972; Admiraal et al., 1982), in many studies diatoms are only investigated in the top few centimetres of the sediment (Riznyk et al, 1978; Colijn & Dijkema, 1981; Shaffer & Onuf, 1983; Colijn & de Jonge, 1984; Varela & Penas, 1985; Lukatelich & McComb,1986). Figure 2 shows that a large potential carbon source may be available far below the photic zone, in spite of low chlorophyll concentrations that are generally recorded at these depths. Thus considerable food is available for infaunal invertebrate grazers and considerable carbon cycling can occur deep in the sediment. Gargas and Gargas (1982) showed that diatoms can still retain a photosynthetic capacity even after several months in darkness. Diatoms in the deeper sediments therefore also form an important pool of potential primary producers which can resume photosynthesis if the surface algae are removed. This factor assumes some importance in the light of sediment turnover rates in Langebaan.

The tidal currents and wind may be responsible for a limited amount of sediment disturbance, but reworking by the local

fauna seems of far greater importance. The sandshark Rhinobatus annulatus and the greater flamingo Phoenicopterus ruber disturb the superficial layers of the sediment, but Branch and Pringle (1987) have demonstrated that Callianassa kraussi is probably the main bioturbator, bringing to the surface up to 12 $kg.m^{-2}.d^{-1}$ of sediment, which is equivalent to a 70% turnover of the top 30 cm in a month. The diatom population of Langebaan lagoon needs to be understood in the light of this continuous sedimentary upheaval.

Diatom biomasses in the lagoon are similar to those of other benthic invertebrates . Mean dry biomass of the intertidal invertebrate macrofauna is 12.6 $g.m^{-2}$ (Carr, unpublished data), while bacterial biomass is about 14.2 $g.m^{-2}$ (Mazure and Branch, 1979). Maximum diatom biomasses at Bottelary and Geelbek were 18.9 and 14.9 $g.m^{-2}$. However, although microalgal biomass appears significant in these terms, a rough estimate of the contribution to the total organic pool, using the correlation between organic material and the percentage mud in the sediment (Mazure and Branch, 1979), and assuming a 70% organic content for diatoms, shows that microalgae constitute a very small fraction (<2%) of the organic pool.

Figure 3 shows the percentage of the diatom biomass composed of pennate diatoms at various stations around Langebaan lagoon (to a depth of 30 cm in the sediment). At sites of high diatom biomass the percentage of pennate diatoms was

FIGURE 3

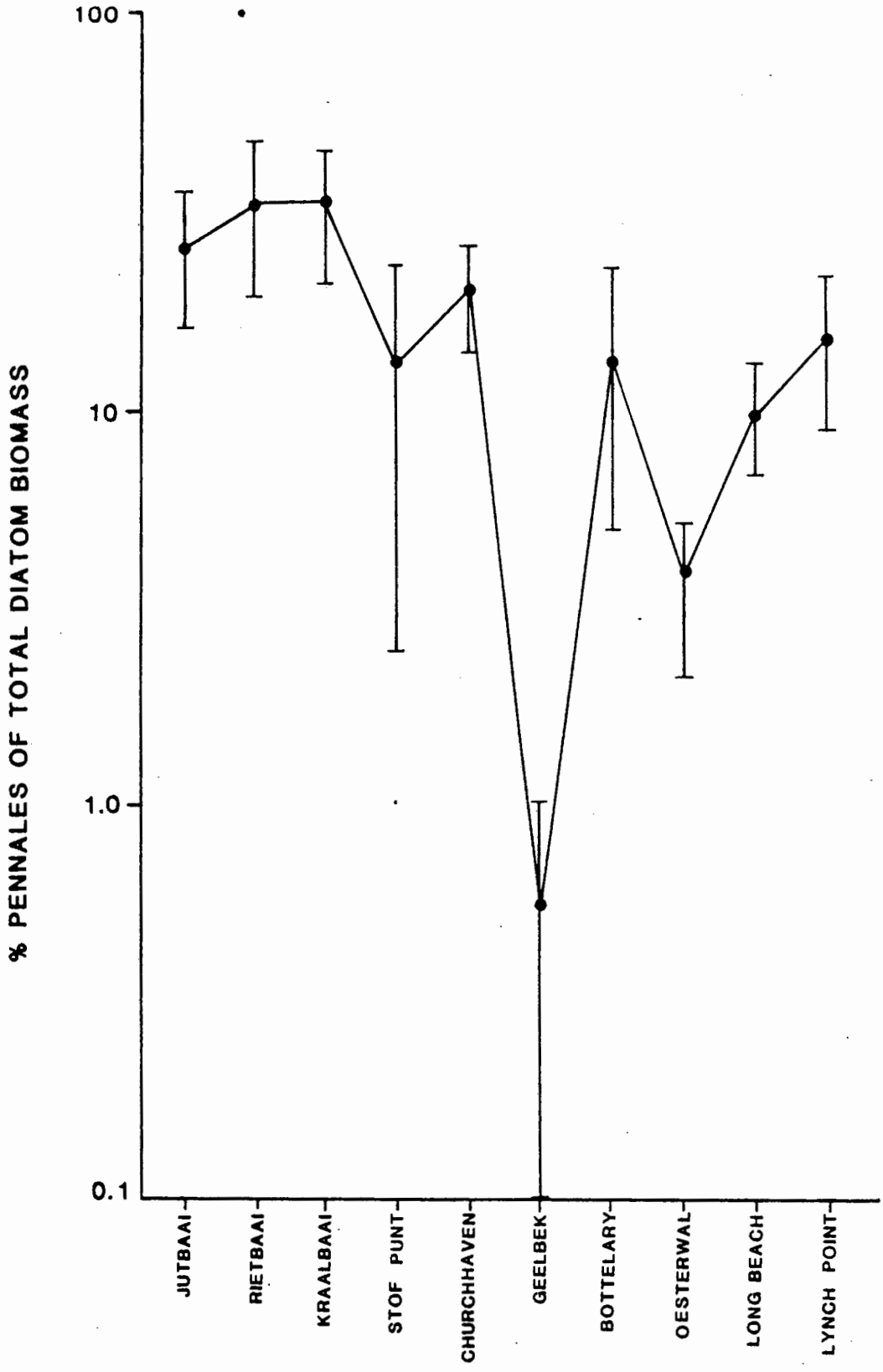


Figure 3. The percentage of total diatom biomass (±S.D.) in the top 30 cm of sediment, which comprised pennate diatoms.

lowest, particularly at Geelbek where current velocity is least, and sediment phi values and the mud fraction are highest. If pennate diatoms are motile and non-pennate diatoms are considered non-motile, then non- motile diatoms predominated at the lowest end of the current and wave energy gradient, presumably because the chances of being washed away were small. At exposed sites and those subjected to stronger currents, motile forms comprised a much larger proportion of the diatom population. The ability to burrow below the surface probably enhances survival when the sediment surface is subjected to waves, and current velocities of 85 to 130 cm. sec^{-1} (Flemming, 1977b).

Sediment chlorophyll.

Sediment chlorophyll concentrations were determined by the method of Lorenzen (1967). Although it has been criticized (Jacobsen, 1978, 1980; Mantoura & Llewellyn, 1983), this method of measuring "active" chlorophyll *a* is still widely used because of its simplicity and applicability to processing large numbers of samples (see Colijn & Dijkema, 1981; Davis & McIntire, 1983; Rasmussen *et al*., 1983; Colijn & de Jonge, 1984; Nienhuis & de Bree, 1984 Lukatelich & McComb, 1986). However it does have certain drawbacks because it does not distinguish between active chlorophyll and degradation products such as chlorophyllide *a*, which may constitute a significant proportion of the chlorophyll fraction that is converted to phaeopigments upon acidification. Chlorophyllase activity, which converts

chlorophyll a to chlorophyllide a, is particularly vigorous in diatoms (Barret & Jeffrey, 1964). Thus spectrophotometric analyses of sediment chlorophyll a are probably over-estimates. The degree of over-estimation appears to vary and depends to some extent on the spectrophotometric method used. Jacobsen (1978) and Mantoura and Llewellyn (1983) obtained over-estimates of up to a factor of 12 when comparing sediment chlorophyll a concentrations determined by trichromatic equations and high pressure liquid chromatography, but Daemen (1986) showed that the Lorenzen method over-estimated sediment chlorophyll a concentrations by approximately 20% when compared with HPLC estimates. Lukatelich and McComb, (1986) showed that chlorophyll a concentrations determined by the Lorenzen method decreased by less than 10% after hexane partioning. In the water column of the kelp beds along on the South African west coast, trichromatic equations significantly over-estimated chlorophyll a concentrations when compared with HPLC determinations, but there was no significant difference between HPLC and Lorenzen determinations (Fielding, 1987). Thus the Lorenzen method appears to provide a reasonable estimate of chlorophyll a concentrations.

Figure 4 shows chlorophyll a concentrations at high, mid and low tide levels for the 1 mm surface layer and at depths of 5, 15 and 25 cm in the sediment at Langebaan lagoon. Ninety six percent of samples had no chlorophyll b, indicating a very minor blue-green or euglenoid algal presence. By far the

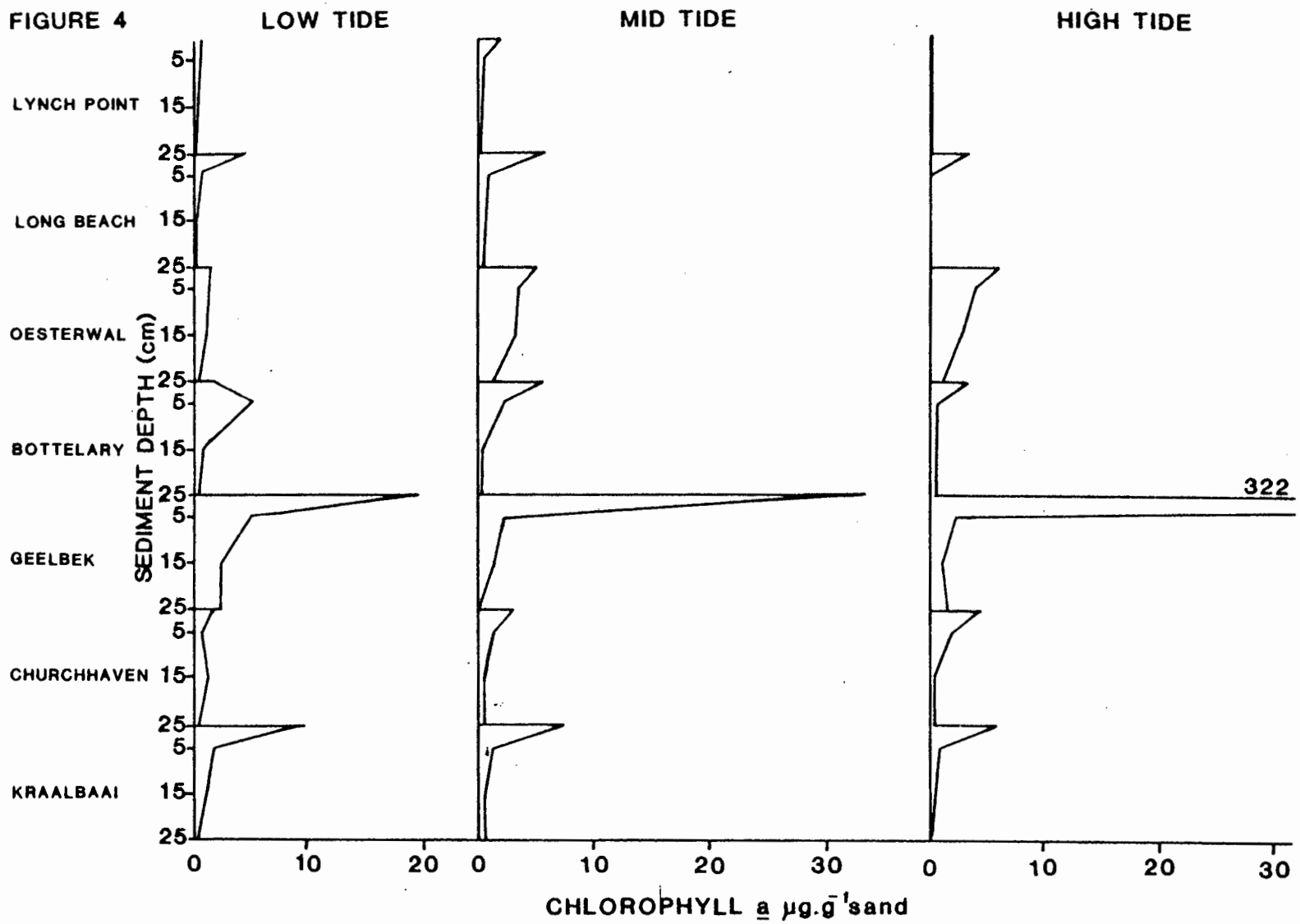


Figure 4. The chlorophyll a content of the sediment at the surface and 5, 15 and 25 cm below the sediment surface, in relation to tidal level.

highest concentrations of chlorophyll *a* (up to 322 µg Chl *a*.g^{-1} sand) occurred at the south-eastern end of the lagoon at Geelbek, which is at the lowest end of the current energy gradient. Very low chlorophyll *a* concentrations (0.49 to 1.03 µg Chl *a*.g^{-1} sand) were found at the exposed Lynch Point. The chlorophyll *a* concentrations at the mid and high tide sites at Geelbek were particularly high because of a microalgal bloom on the sediment surface.

Except for one site, the highest concentrations of chlorophyll *a* occurred in the top millimetre of sediment (Figure 4). Chlorophyll *a* concentrations 5 cm below the sediment surface were usually between 16% and 86% of the corresponding surface layer concentrations with the mean value being 35% (SD 26.3). Generally chlorophyll concentrations were even less at 15 and 25 cm below the sediment surface. However Figure 2 indicates that a very considerable diatom biomass exists at depths of 10-20 cm and 20-30 cm. This underlines the discrepancies that can occur when biomass is assessed only from chlorophyll *a* concentrations in the sediment. The decline in chlorophyll *a* concentrations with depth, indicates that there is a reduction in the chlorophyll *a* content of the diatoms after burial (see also Gargas & Gargas, 1982).

Figure 4 shows no consistent gradient in sediment chlorophyll *a* concentrations from high tide to low tide or vice versa. To assess the standing stocks more accurately, the data on

chlorophyll *a* concentrations. g^{-1} sediment were converted to chlorophyll *a*.cm^{-3} sediment using weight:volume conversions, and integrated over 25 cm. Despite this conversion there was no consistent pattern in the distribution of sediment chlorophyll *a* relative to tidal height. A one way analysis of variance of the percentage chlorophyll *a* in the top millimeter at high, mid and low tide levels also showed no difference between the tidal levels ($F = 1.54$, d.f. = 18, $p > 0.05$).

The percentage of phaeopigments in the chlorophyll *a* samples is shown in Figure 5. It is evident that the pigments in the top millimetre of sediment were almost entirely active chlorophyll *a* and the level of phaeopigments was very low. Below this there was a sharp increase in the ratio of phaeopigment to chlorophyll *a*, usually progressively rising with depth. In many cases phaeopigments comprised 50% or more of the pigments assayed: similar values have been reportd by Cadee (1980) and Varela and Penas (1985). This indicates that much of the pigment in the deeper sediments is non-functional, and stresses the importance of distinguishing between functional chlorophyll *a* and phaeopigments in tidal flat sediments. This is in contrast to the findings of Steele and Baird (1968), who showed no great increase in chlorophyll *a* breakdown products with increasing depth in the sand. Riznyk and Phinney (1972) detected significant differences in the percentage of phaeopigments from lower, mid and upper

FIGURE 5

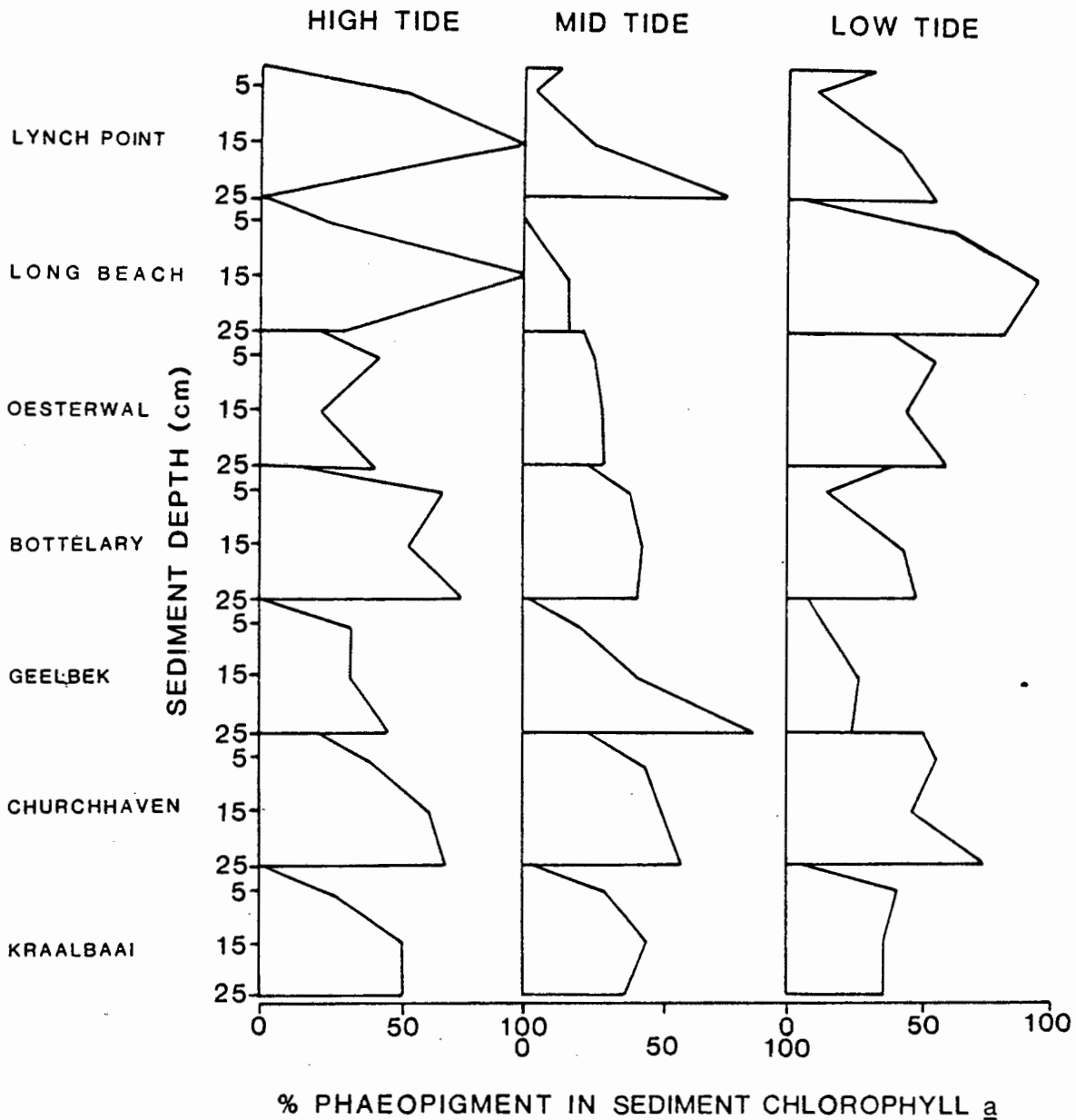


Figure 5. The percentage of phaeopigments in sediment chlorophyll a at the surface and 5, 15 and 25 cm below the sediment surface at various stations around Langebaan lagoon.

intertidal zones, with higher percentages being recorded from the upper intertidal area. No such trend is apparent in our data.

At the time measurements of carbon fixation were made at four stations on the north eastern side of the lagoon (in June 1980), chlorophyll a concentrations in the top millimetre of sediment (Fig. 6) were similar to those recorded a year previously (Figure 4), except that the values for Geelbek were much lower, since the yellow-brown bloom noted in the previous year was absent during the latter experiments. Highest chlorophyll a concentrations (19.7 $ug.g^{-1}$ sand) were again recorded from Geelbek, and chlorophyll a concentrations in both the 1 mm and 2-5 mm layer of sediment increased with declining exposure to wave action and current energy, from Long Beach to Geelbek. Using appropriate weight:volume conversions, chlorophyll a concentrations were integrated over 5 mm, and when expressed per unit area show a very distinct increase from the exposed northern to the more sheltered southern end of the lagoon (Figure 6). No phaeopigments were found in the samples, again reflecting the fact that most of the pigment in the shallow surface layers of the sediment is functional chlorophyll.

Carbon fixation

Figure 6 shows the rate of carbon fixation by benthic microalgae in the top 5 mm of sediment at the four stations

FIGURE 6

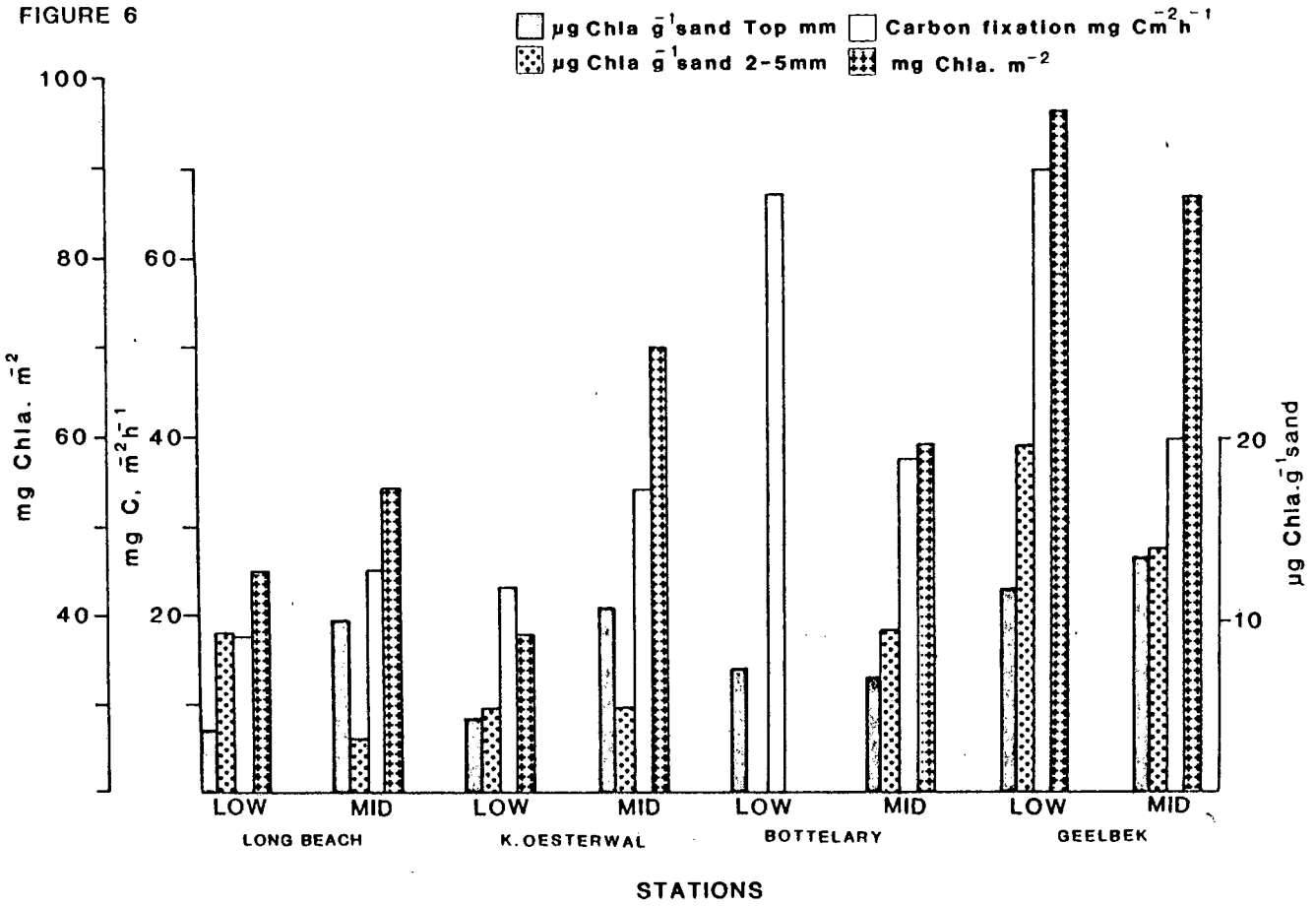


Figure 6. Carbon fixation in the top 5 mm of sediment, and chlorophyll a content at the surface and 2-5 mm below the surface at two tidal levels and four stations around Langebaan lagoon. Chlorophyll a integrated over 5 mm of sediment is also shown.

(Long Beach, Klein Oesterwal, Bottelary and Geelbek, see Figure 1). Carbon production values ranged from 17.38 mg $C.m^{-2}.h^{-1}$ at Long Beach to 69.54 mg $C.m^{-2}.h^{-1}$ at Geelbek. Phytoplankton production is not greatly in excess of this. The data of Henry et al. (1977) show that the peak production of phytoplankton occurred in spring and averaged 100 mg $C.m^{-2}.h^{-1}$ for the whole lagoon, but by summer production in the southern reaches was reduced to less than 50 mg $C.m^{-2}.h^{-1}$, and south of Bottelary, in the more sheltered portion of the lagoon, production was less than this (Henry et al., 1977). Christie (1981) measured phytoplankton primary production at depths of 1, 2 and 3 m at eight stations in the channel between Rietbaai and Geelbek. Production was highest in summer and at the exposed northern end of the lagoon (300 to 314 mg $C.m^{-3}.day^{-1}$) and lowest in winter and at the sheltered southern end of the lagoon (40 to 50 mg $C.m^{-3}.day^{-1}$). Most of the lagoon is less than 3 m deep (Flemming, 1977a), so for purposes of comparison the production figures of Christie (1981) were converted to production per m^2 assuming a 3 m depth and a 12 hour photoperiod (Henry et al., 1977). Production is thus approximately 75 to 78 mg $C.m^{-2}.h^{-1}$ in summer in the vicinity of Long Beach/Klein Oesterwal, and 10 to 12 mg $C.m^{-2}.h^{-1}$ in winter at Geelbek. These values are similar to, but slightly lower than, those of Henry et al., (1977). Christie (1981) measured primary production directly while Henry et al., (1977) calculated primary production indirectly from chlorophyll a concentrations. When these values are compared

with the benthic carbon fixation rates in Figure 6, it is evident that the productivity.m^{-2} of the lagoonal benthic microalgae is probably at least as great as that of the phytoplankton, in spite of the fact that the productivity of phytoplankton is integrated over an average water depth of 3 m in comparison to the 5 mm of sediment for which benthic production was calculated. In addition, the benthic carbon fixation rates were determined in winter. Since temperature and light intensity are the most important factors influencing carbon fixation by benthic microalgae, and increases in microfloral production rates have been recorded in spring and summer months elsewhere (Cadee & Hegeman, 1974; Dye, 1978; Cadee, 1980; Davis & McIntire, 1983; Schaffer & Onuf, 1983; Colijn & de Jonge, 1984; Varela & Penas, 1985), it is probable that benthic primary production rates at Langebaan lagoon will also increase in spring and summer and may exceed phytoplankton production rates.

Phytoplankton production decreases from north to south in Langebaan lagoon together with a decline in nitrates (Henry et al., 1977; Christie, 1981), whereas the opposite trend is apparent in benthic primary production. This suggests that the benthic microflora is independant of nutrient concentrations in the water column, relying rather on nutrients recycled in the sediment, while the large macrophyte population in the southern reaches of the lagoon (Christie, 1981) may rapidly deplete water column nutrients

that are introduced each tidal cycle, and so reduce phytoplankton productivity.

Chlorophyll a content of the sediment can be measured quickly and accurately and large numbers of samples can be analysed to give an overall picture of standing stocks of benthic microalgae. Production measurements are not always readily undertaken, and as a guide a conversion factor can be derived from our data (Figure 6). Only the top 5 mm of sediment were considered, within which all primary production must occur and where phaeopigments were absent. A regression of chlorophyll a against benthic production yields the equation $Y = 25.68 + 1.098X$ ($r^2 = 0.87$, n=7), where Y is chlorophyll a concentration (mg Chl a.m^{-2}) and X is carbon fixation (mg C.m^{-2}.h^{-1}). It is recognized that this quantification is at best a rough one since benthic primary production may be seasonal while sediment chlorophyll levels may not always exhibit the same seasonality (Pamatat, 1968; Riznyk & Phinney,1972; Cadee & Hegeman, 1974; Riznyk et al., 1978; Varela & Penas, 1985; Lukatelich & McComb, 1986). Therefore ratios of chlorophyll a : carbon uptake may vary. Also photosynthetic and respiration rates of benthic diatoms may exhibit a tidal rythmn (Pamatat, 1968). However, Davis and McIntire (1983) and Colijn and de Jonge (1984) have found a significant positive correlation between sediment chlorophyll a concentrations and primary production, so the relationship calculated above does provide a guide to the relative importance of benthic primary production in lagoons and

estuaries in the Cape Province of South Africa. Further work is planned to determine the seasonal variability of the relationship.

Relative productivity of benthic microalgae

Using the mean daily photoperiod of 12 hours estimated by Henry et al. (1977) for Langebaan lagoon, an approximate annual benthic microalgal production can be calculated. This yields figures of 63 g $C.m^{-2}.y^{-1}$ for the sandy Long Beach and 253 g $C.m^{-2}.y^{-1}$ for the mud and sand at Geelbek. These values agree well with those obtained by other workers in a variety of localities (Pamatat, 1968; Cadee & Hegeman, 1974; Riznyk & Phinney, 1978; Cadee, 1980; Davis & McIntire, 1983; Nienhuis & de Bree, 1984; Varela & Penas, 1985). Dye (1978) records production rates of 47.0 g $C.m^{-2}.y^{-2}$ in autumn and 58.5 g $C.m^{-2}.y^{-2}$ in spring on a sandy beach, and 109.5 g $C.m^{-2}.y^{-1}$ (autumn) and 123.0 g $C.m^{-2}.y^{-1}$ (spring) on a muddy beach, in the Swartkops estuary in South Africa. These are very similar to values obtained in this study.

Some rough comparisons may be made between benthic diatom production and the production of macrophytes and phytoplankton in Langebaan lagoon (Table 1).

Macrophyte production in Langebaan lagoon has been investigated by Christie (1981). Sarcocornia perennis, Spartina maritima and Gracilaria verrucosa are the most important macrophytes in the system. Sarcocornia has the

TABLE 1.

Comparison of primary production by macrophytes, phytoplankton and benthic diatoms

	Annual Production $kJ.m^{-2}$	Calorific value $kJ.g^{-1}C$	Annual Production $g\ C.m^{-2}$	Area ha.	Annual Production $g\ C.y^{-1}$	% of total
Sarcocornia	31×10^3 (a)	45 (b)	689	955 (a)	6.6×10^9	35
Spartina	20×10^3 (a)	40 (c)	500	257 (a)	1.3×10^9	7
Gracilaria	17×10^3 (a)	46 (d)	370	342 (a)	1.3×10^9	7
4 species minor macrophytes	6×10^3 - 52×10^3 (a)	40-45 (e)	133-1300	6-275 (a)	1.1×10^9	6
Total for macrophytes		-	-	-	10.3×10^9	55
Phytoplankton			123	3530 (f)	4.3×10^9	23
Benthic diatoms			171	2350 (g)	4.0×10^9	22
				Total	18.6×10^9	100

Footnotes
(a) (Christie, 1981).
(b) No value available: mean value of understorey algae in kelp bed used (Newell et al., 1982)
(c) (Pierce, 1979).
(d) (Harris, 1979, unpublished report).
(e) No values available: mean value for understorey algae in kelp bed (Newell et al., 1982) used for algal macrophytes and Spartina value (Pierce, 1979) used for angiosperm macrophytes.
(f) (Christie and Moldan, 1977; Fleming, 1977b).
(g) (Summers, 1977).

highest annual production of 31 x 10^3 kJ.m^{-2} and Spartina and Gracilaria have annual productions of 20 x 10^3 kJ.m^{-2} and 17 x 10^3 kJ.m^{-2} respectively. For comparison with data from this study, these values were converted to g C.m^{-2} by means of calorific and CHN values obtained by other workers (see Table 1).

If benthic diatom production rates from all locations shown in Figure 6 are averaged (39.09 mg C.m^{-2}.h^{-1}) and a 12 hour photoperiod assumed (Henry et al., 1977), annual benthic diatom production is 171 g C.m^{-2}. This is somewhere between net and gross production (Steele & Baird, 1968). The mean winter and summer phytoplankton production from all 8 stations of Christie (1981) was 112 mg C.m^{-3}.day^{-1} which, when integrated over the mean lagoon depth and calculated on an annual basis, amounted to 123 g C.m^{-2}.y^{-1}. In terms of unit area, macrophytes are therefore the most productive primary producers in Langebaan lagoon with values ranging from 138 to 688 g C.m^{-2}.y^{-1} (or even 1300 g C.m^{-2}.y^{-1} if the upper value is accepted for the minor macrophytes). The production of benthic diatoms (171 g C.m^{-2}.y^{-1}) and phytoplankton (123 g C.m^{-2}.y^{-1}) is approximately equal and much lower than that of the principal macrophytes. However it is important to consider the area available for each kind of primary production.

When the area occupied by each species is taken into account, total production of the lagoon's macrophytes is about

10.3×10^9 g $C.y^{-1}$ (Table 1). The lagoon has 1750 hectares of intertidal sand flats and 600 hectares of saltmarshes (Summers, 1977). Assuming a benthic diatom production of 171 g $C.m^{-2}.y^{-1}$ occurs throughout the saltmarshes and sandflats, benthic diatoms produce approximately $4,0 \times 10^9$ g $C.y^{-1}$. Mean phytoplankton production was 123 g $C.m^{-2}.y^{-1}$. The total volume of the lagoon at spring low tide is 7.1×10^7 m^3 (Christie & Moldan, 1977). Approximately 7×10^7 m^3 water flows in and out of the lagoon at each tidal cycle (Flemming, 1977b), so at high tide the lagoon contains approximately 14×10^7 m^3 water. An "average" lagoon volume would be low tide volume plus half the volume that flows in and out at each tidal cycle (ie. 10.6×10^7 m^3). Assuming a mean depth of 3m, this volume of water has a surface area of 3530 ha and total phytoplankton production will be 4.3×10^9 g $C.y^{-1}$. It is clear that in terms of the total primary production of carbon in Langebaan lagoon, the macrophytes are the most important contributers in the lagoon ecosystem. However, benthic diatoms contribute 22% of the carbon budget, and any interference with the tidal flats by, for example development or pollutants, will have a considerable impact on the system.

Acknowledgements

The authors thank Pete Ryan and Nadine Zambetti for assistance with diatom counts and field work, Dr M. Lucas for critical reading of the manuscript, Di Gianakouras for production of the figures and Sandy Tolosana for preparation of the manuscript. This work was supported by a grant from the South African Council for Scientific and Industrial Research.